

Hypothesis: *Musculin* is a hormone secreted by skeletal muscle, the body's largest endocrine organ.

Evidence for actions on the endocrine pancreas to restrain the β -cell mass and to inhibit insulin secretion and on the hypothalamus to co-ordinate the neuroendocrine and appetite responses to exercise

Dennis Engler

Division of Endocrinology and Metabolism, Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia

Abstract. Recent studies indicate that skeletal muscle may act as an endocrine organ by secreting interleukin-6 (IL-6) into the systemic circulation. From an analysis of the actions of IL-6 and of additional literature, we postulate that skeletal muscle also secretes an unidentified hormone, which we have named *Musculin* (*Latin: musculus = muscle*), which acts on the pancreatic β -cell to restrain the size of the β -cell mass and to tonically inhibit insulin secretion and biosynthesis. It is suggested that the amount of *Musculin* secreted is determined by, and is positively correlated with, the prevailing insulin sensitivity of skeletal muscle, thereby accounting for the hyperinsulinemia that occurs in insulin resistant disorders such as type 2 diabetes mellitus, obesity, and the polycystic ovary syndrome. In addition, it is postulated that *Musculin* acts on the hypothalamus (arcuate nucleus, dorsomedial hypothalamic nucleus) to co-ordinate the neuroendocrine and appetite responses to exercise. However, the possibilities that *Musculin* may act on additional central nervous system sites and that an additional hormone(s) may be responsible for these actions are not excluded. It is suggested that a search be made for *Musculin*, since analogues of such a substance may be of therapeutic benefit in the treatment of the current global diabetes and obesity epidemic. (www.actabiomedica.it)

Key words: *Musculin*, skeletal muscle hormone, exercise, pancreatic beta cell, hypothalamus, growth hormone, adrenocorticotropin, prolactin

Introduction

The concept that skeletal muscle may act as an endocrine organ has received credence from studies of the effects of exercise on plasma concentrations of interleukin-6 (IL-6) and IL-6 gene expression in skeletal muscle. IL-6 is a member of a family of cytokines that share a similar helical protein structure and a similar receptor subunit (1). IL-6 exerts its cellular effects by binding to membrane-bound or soluble IL-6 receptors and the liganded receptor(s) then associate(s) with the membrane-bound glycoprotein gp130 (2). The IL-6r-gp130 heterodimer activates members of the Janus-activated protein kinases (JAKs) which

then phosphorylate and activate the Signal Transducer and Activator of Transcription (STAT-3) in many cell types. As a result of STAT-3 activation, IL-6 activates a family of proteins including the Suppressor of Cytokine Signaling (SOCS) protein, SOCS-3 (3).

IL-6 is produced by cells of the reticuloendothelial and immune systems as well as keratinocytes, osteoblasts, adipose tissue, smooth muscle and skeletal muscle cells (1,4). Although skeletal myocytes produce IL-6 in response to those inflammatory stimuli that also release the cytokine from monocytes and cardiac myocytes, skeletal myocytes appear unique in their ability to release IL-6 in response to muscle contraction and in the absence of inflammation (5). The con-

traction-induced rise in IL-6 gene transcription seems predominantly localized to the type 2 fibers and may be mediated by a rise in cytosolic Ca^{2+} that occurs during the contractile process (6).

During exercise, glucose disposal increases but hypoglycemia is prevented by a concomitant increase in hepatic glucose production (HGP). The increased HGP that occurs during exercise of moderate intensity is thought to be mainly due to an increased portal venous glucagon:insulin ratio, although exercise of more severe intensity also stimulates the secretion of growth hormone (GH), epinephrine (EPI), and cortisol. Since the time course with which these counterregulatory factors increase cannot account for the rapid exercise-induced increase in HGP, it has long been suspected that an as yet unidentified factor released from skeletal muscle might contribute to the increased HGP. It now appears that IL-6 may partly, or wholly, fulfil the criteria of this so-called “work factor” (Figure 1) and since plasma levels of IL-6 positively correlate with exercise intensity, IL-6 may become an important stimulus of HGP as exercise intensity increases (7-12).

IL-6 acts as an insulin antagonist in the liver by inhibiting glycogen synthase activity and accelerating glycogen phosphorylase activity (13). The cytokine al-

so induces SOCS-3 expression in the liver *in vitro* and *in vivo* which inhibits hepatic insulin receptor autophosphorylation, insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation, the association of IRS-1 with the p85 subunit of phosphatidylinositol (PI) 3-kinase and activation of serine/threonine protein kinase Akt (14). By contrast, when IL-6 is depleted in the leptin-deficient *ob/ob* mouse by immunoneutralization with an IL-6 antibody, hepatic insulin sensitivity is selectively increased (15). Although IL-6 acts as an insulin antagonist in the liver, it acts as an insulin sensitizer in skeletal muscle by enhancing the ability of insulin to stimulate muscle glycogen synthesis. These findings indicate that IL-6 exerts tissue-specific effects on insulin action (16).

Skeletal muscle structure

The aforementioned studies therefore assign to skeletal muscle the status of an endocrine gland and, given its sheer size, it would appear to be the largest of its kind in the body. Skeletal muscle contains a large number of genes whose expression is regulated by that powerful modulator of muscle insulin sensitivity, physical exercise (17-28). In this section, we provide a brief outline of the major components of skeletal muscle in order that the reader may obtain a glimpse of the mechanisms by which muscular contraction may modify skeletal muscle gene expression (29-35).

The characteristic striated appearance of myofibrils as alternating light (I-band) and dark (A-band) bands results from the precise alignment of the filament systems of the sarcomere, the basic contractile unit of the myofibrils. The sarcomere is principally composed of parallel arrays of actin-containing thin filaments, the thick myosin-containing filaments, single titin molecules, and the giant protein, nebulin (Figure 2). The actin molecules have been implicated in diverse cellular functions such as motility, cytokinesis, and contraction and are anchored in the Z-disc and span the I-band. The I-region links the A-band, the region of active force generation, with the bordering Z-lines, and also contains part of the immense protein, titin. The actin filaments extend toward the middle of the sarcomere and, in the A-band, they interdigitate with the myosin-containing thick fila-

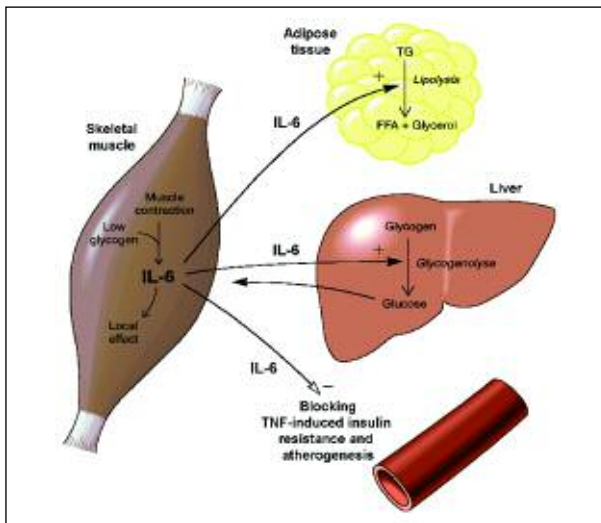


Figure 1. Schematic presentation of the biological effects of muscle-derived IL-6. TG, triglyceride; FFA, free fatty acid; TNF, tumor necrosis factor. (reproduced with permission from ref. 4)

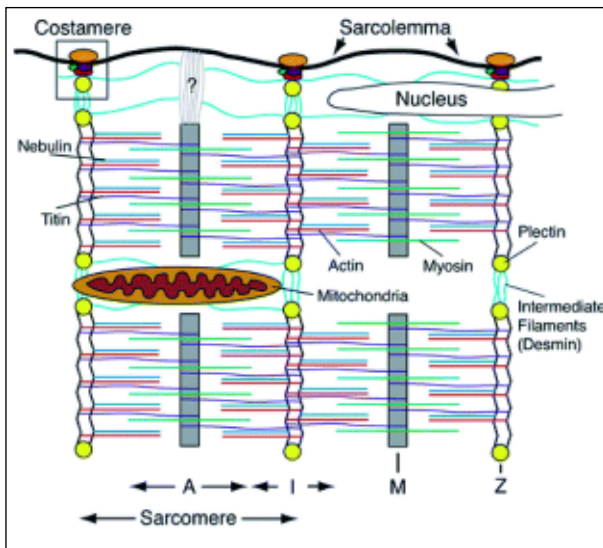


Figure 2. A schematic overview of cytoskeletal linkages in striated muscle. The sarcomeres contain four filament systems: actin-thin, myosin-thick, titin, and nebulin filaments. The borders of individual sarcomeres are the Z-lines, which are precisely aligned and laterally associated with intermediate filament proteins (such as desmin) and other cytoskeletal proteins (such as plectin). The intermediate filaments and associated proteins also may link the peripheral myofibrils to costameres at the sarcolemma (the muscle membrane), to mitochondria, and to the nuclear membrane. Although many of the detailed interactions are not yet known, these linkages are responsible for the mechanical integration and stability of myofibrils, organelles, and membrane components for effective force transmission. The microtubule system is not depicted in the schematic because it is unclear how they are arranged in striated muscle; however, they may be linked to myofibrils and intermediate proteins such as plakin family members (reproduced with permission from ref. 32)

ments. The M-line region is the anchoring site for the thick filaments and its appearance is considered to be the final step in myofibril assembly. The Z-lines define the lateral boundaries of the sarcomere, they are the anchoring sites for the thin, titin, and nebulin filaments, and are thus the primary means of transmission of the force generated by contraction.

The third filament system is made up of the huge modular protein, titin. The N-terminal ends of titin overlap in the Z-line, the titin molecules span the I- and A-bands and their C-terminal ends overlap in the M-line, thus forming a continuous filament system in the myofibrils. Titin possesses several distinct properties—first, it may function as a molecular spring

and thus determine myofibrillar stiffness; second, titin contains repeating motifs, it is assembled early in myofibrillogenesis and it interacts with several sarcomeric components, and may therefore stabilize the sarcomere; third, the titin C-terminal region contains a serine-threonine kinase domain which has been recently shown to control muscle gene expression and protein turnover (34). These findings provide a structural basis by which physical exercise may modify skeletal muscle gene expression, including key proteins of the insulin signaling pathway (Figure 3).

The fourth filament system is made up of another giant protein, nebulin, which spans the length of the actin filaments. The C-terminal end of nebulin is partially inserted into the Z-lines whereas its N-terminal end extends to the ends of the thin filaments. Nebulin is inextensible and may therefore specify the

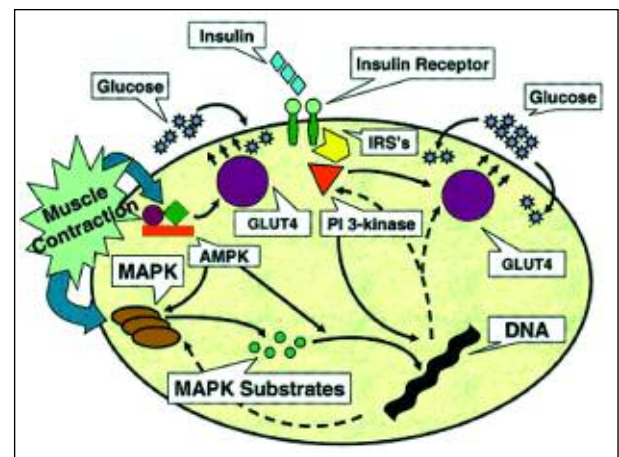


Figure 3. Exercise training-induced changes in insulin signaling in skeletal muscle. Insulin signal transduction through the insulin receptor, insulin receptor substrate (IRS)-1/2 and phosphatidylinositol 3-kinase (PI3-kinase) is enhanced in skeletal muscle in the hours after an exercise bout. These changes may enhance insulin sensitivity, as well as regulate gene expression after exercise. Immediately after exercise, mitogen-activated protein kinase (MAPK) signaling to downstream substrates is enhanced, providing a possible molecular mechanism for exercise-induced transcriptional regulation in skeletal muscle. Acute exercise also increases AMP-activated protein kinase (AMPK) activity, leading to changes in glucose uptake and gene expression. Exercise training is associated with changes in mRNA of several components of insulin and MAPK signaling cascades. The “master regulator(s)” of exercise-responses on gene expression has not been completely defined (reproduced with permission from ref. 61)

precise lengths of the thin filaments. Since physical exercise regulates skeletal muscle genes that code for proteins of both known and unknown functions, we postulate that:

- One of these unknown transcripts codes for a hormone which we have named *Musculin*.
- The amount of *Musculin* secreted is determined by, and is positively correlated with, the prevailing insulin sensitivity in skeletal muscle.
- *Musculin* acts on the endocrine pancreas to restrain the overall size of the β -cell mass and to tonically inhibit the β -cell's capacity to synthesize and secrete insulin.

These postulates are depicted schematically in Figure 4 and are discussed below.

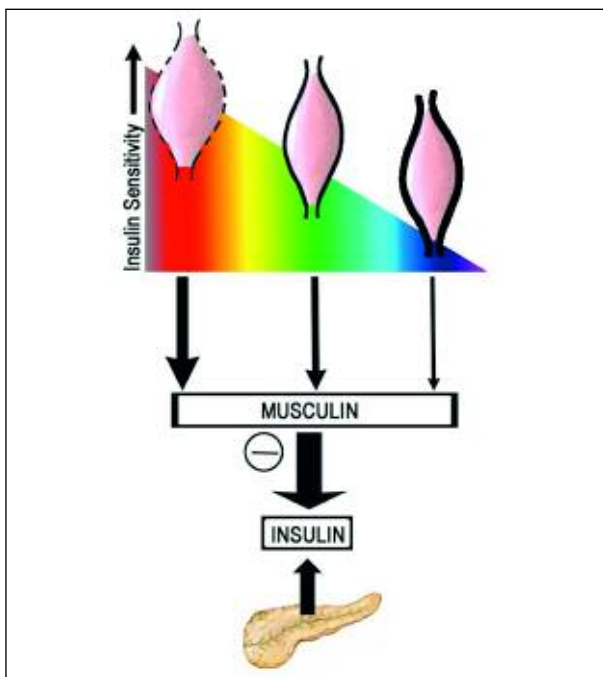


Figure 4. Schematic representation of the secretion of *Musculin* as a function of muscle insulin sensitivity. It is proposed that *Musculin* is a hormone released by skeletal muscle that acts on the pancreatic β cell to restrain β -cell mass and β -cell secretion of insulin. Furthermore, it is suggested that the amount of *Musculin* secreted is determined by, and positively correlated with, muscle insulin sensitivity. The lines around the periphery of each muscle designate insulin sensitivity. *Left*, increased insulin sensitivity (---); *Centre*, normal insulin sensitivity (—); *Right*, reduced insulin sensitivity (—). The width of the arrows leading from the skeletal muscles to the pancreas schematically depict the amount of the hormone *Musculin* that is secreted

Evidence that skeletal myocytes contain the intracellular machinery required for hormone secretion

The suggestion that skeletal muscle may function as an endocrine organ presupposes that the myocyte is capable of transporting hormones from their intracellular site of synthesis to the cell surface and of secreting these hormones into the systemic circulation. Intracellular proteins that are destined for secretion are usually transported to the cell surface in vesicles and this process has been most intensively investigated in presynaptic nerve terminals (36-39). A brief outline of the proteins involved in this 'Synaptic Vesicle Cycle' and a description of their skeletal myocyte counterparts is provided below.

When an action potential causes the opening of Ca^{2+} channels in a nerve terminal, the resulting Ca^{2+} transient stimulates synaptic vesicle exocytosis and neurotransmitter release (Figure 5, ref. 39). The steps in the trafficking cycle for synaptic vesicles can be enumerated as follows: (Step 1) Neurotransmitters are actively transported into synaptic vesicles and (Step 2) cluster in front of the active zone. They then dock at the active zone (Step 3), where they are primed (Step 4) to render them competent for Ca^{2+} -triggered fusion-pore opening (Step 5). The synaptic vesicles may be recycled by either of two fast pathways or one slower pathway. The fast pathways are preferentially used when the frequency of nerve stimulation is low, during which the vesicles either remain at the active zone and are refilled, or are locally recycled without clathrin-mediated endocytosis. The slower pathway involves clathrin-mediated endocytosis and is utilized at higher frequencies of nerve stimulation. The process of membrane fusion involves SNARE proteins that are characterized by an homologous 70-residue sequence termed the SNARE motif. The SNARE proteins are present on both fusing membranes before fusion and they associate into tight core complexes during fusion. Vesicle endocytosis is mediated by three SNARE proteins: i) Synaptobrevin (or Vesicle-Associated Membrane Protein, VAMP) on the synaptic vesicle, (ii) Syntaxin 1 and (iii) SNAP-25 located on the presynaptic cell membrane (Figure 6).

The scheme shown in Figure 6 proposes that the SNARE complex pulls the synaptic vesicle and plasma

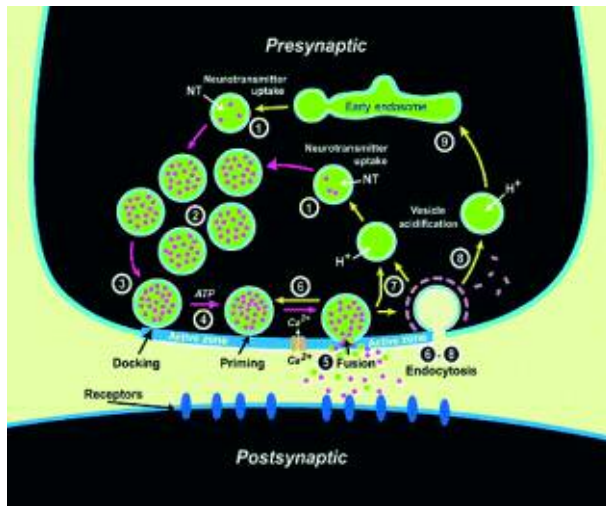


Figure 5. The synaptic vesicle cycle. Synaptic vesicles are filled with neurotransmitters by active transport (step 1) and from the vesicle cluster that may represent the reserve pool (step 2). Filled vesicles dock at the active zone (step 3), where they undergo a priming reaction (step 4) that makes them competent for Ca^{2+} triggered fusion-pore opening (step 5). After fusion-pore opening, synaptic vesicles undergo endocytosis and recycle via several routes: local reuse (step 6), fast recycling without an endosomal intermediate (step 7), or clathrin-mediated endocytosis (step 8) with recycling via endosomes (step 9). Steps in exocytosis are indicated by red arrows and steps in endocytosis and recycling by yellow arrows (reproduced with permission from ref. 39)

membranes close together and creates an unstable intermediate. The intermediate can either progress to a fusion pore or regress to the docked state of synaptic vesicles that do not contain engaged SNAREs. Complexins may then bind and stabilize the synaptic core complex which is essential for the proper positioning of synaptotagmin 1.

Synaptotagmins 1 and 2 are abundant synaptic vesicle proteins that act as Ca^{2+} sensors for fast exocytosis. Synaptotagmin 1 binds to the SNARE complex in the absence of Ca^{2+} , but switches to binding the phospholipid membrane when Ca^{2+} enters. This may then destabilize the fusion intermediate and open the fusion pore. Synaptotagmin 1 is part of a gene family containing 15 members, and it is possible that one or more of these other family members mediates Ca^{2+} -induced slow exocytosis. SNARE complex formation is also regulated by SM (Sec1/Munc18-like) proteins, tomosyn, amisyndin and the synaptophysins. Synaptophysins are abundant synaptic vesicle proteins that bind synaptobrevin and may restrict its availability for fusion.

These observations are of relevance for skeletal muscle (and adipocyte) function since synaptobrevins 1 and 2, syntaxin 4, and VAMP 2 and 3 have been

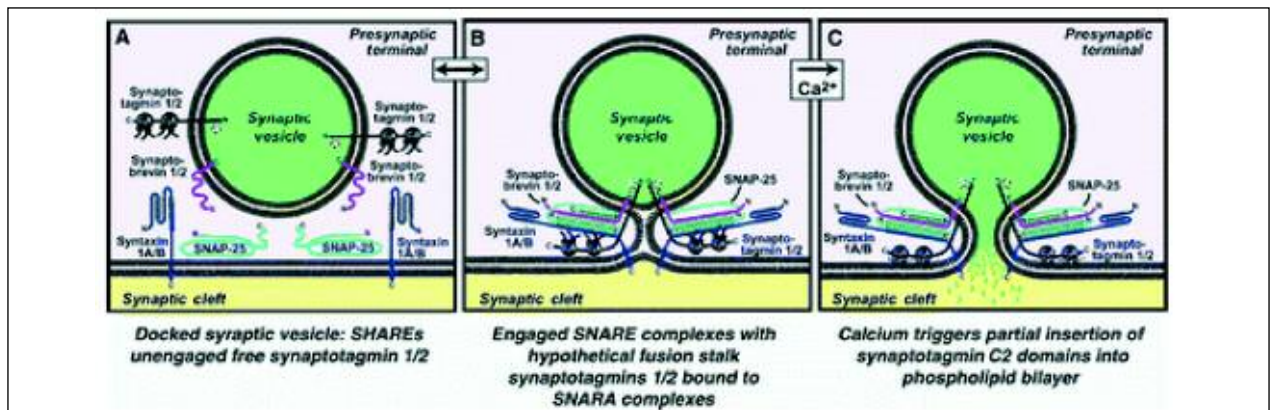


Figure 6. Model for the functions of SNARE proteins, complexes, and synaptotagmins 1 and 2 in synaptic vesicle exocytosis. In docked vesicles (*panel A*), SNAREs and synaptotagmins are not engaged in direct interactions. During priming (*panel B*), SNARE complexes form, complexins (green) are bound to fully assembled complexes, and synaptotagmins constitutively associate with the assembled SNARE complexes. The synaptic vesicle membrane and plasma membranes are forced into close proximity by SNARE complex assembly, which results in an unstable intermediate that is shown as a speculative fusion stalk. Ca^{2+} influx (*panel C*) further destabilizes the fusion intermediate by triggering the C₂ domains of synaptotagmin to partially insert into the phospholipids. This action is proposed to cause a mechanical perturbation that opens the fusion pore. Note that the nature and stability of the putative fusion intermediate is unclear and that SNARE complex assembly in panel B is suggested to be reversible, whereas Ca^{2+} triggering is not (reproduced with permission from ref. 39)

found in skeletal myocytes and adipocytes, and VAMP5 has been isolated from C₂C₁₂ myocytes during myogenesis. The findings therefore suggest that mechanisms for the intracellular trafficking of proteins, similar or identical to those described in synaptic nerve terminals, also exist in skeletal myocytes (and adipocytes, 40-49).

Insulin secretion in states of increased muscle insulin sensitivity

Although an increased sensitivity of skeletal muscle to the actions of insulin can be achieved in the mouse by deletion of the genes coding for the p85 β subunit of phosphoinositide 3-kinase or the ganglioside GM3 (50, 51), the most physiological means of achieving this effect is by physical exercise. Indeed, numerous studies have shown that exercise causes an acute insulin-independent increase in glucose transport which is followed by an increase in skeletal muscle insulin sensitivity (52-65) that is mediated by translocation of more GLUT4 glucose transporters to the myocyte cell membrane (66-77). Exercise acutely reduces insulin secretion (62-64, 78-92) and increases glucagon secretion (93-102) and thus alters the portal venous insulin:glucagon ratio. The rise in portal venous glucagon is essential for the increased gluconeogenesis and HGP and the lowering of portal venous insulin concentration may restrain these effects of glucagon and prevent hyperglycemia.

Several mechanisms may contribute to these exercise-induced changes in islet hormone secretion, and one of these has been thought to be an exercise-induced alteration in autonomic nervous system function. The endocrine pancreas is innervated and regulated by both the parasympathetic nervous system derived from neurons in the dorsal motor nucleus of the vagus (DMV) and the sympathetic nervous system derived from cell bodies in the intermediolateral column (IML) of the spinal cord. Furthermore, these cell groups receive either direct or indirect inputs from second- and third-order neurons located in the prefrontal, piriform and gustatory cortices and several sub-cortical brain areas (Figure 7; 103-111). The parasympathetic cholinergic nerve fibers innervating the islets are postganglionic in origin, they originate from

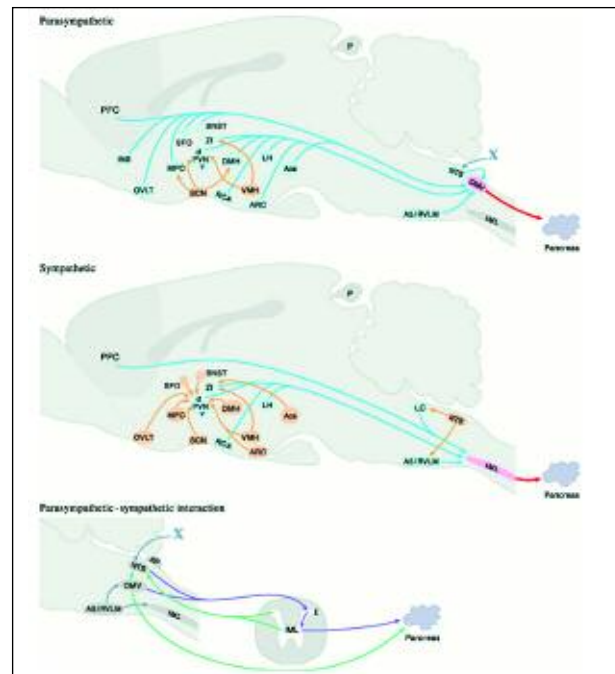


Figure 7. Sagittal scheme of first-order projections to the pancreas (in red), second-order in blue, and third-order in yellow. It is clear by comparing the parasympathetic pattern against the sympathetic pattern that far more second-order cell groups are in control of the dorsal motor nucleus of the vagus than in control of the sympathetic neurons. The parasympathetic-sympathetic interaction illustrates the relationship between the cell groups that may influence the vagal output in green or the sympathetic output in pink. It is clear that both vagal and sympathetic output are influenced by each other. Abbreviations: A5/RVLM, rostral ventrolateral medulla; Ace, amygdala central part; AP, area postrema; ARC, arcuate nucleus; BNST, bed nucleus of the stria terminalis; DMH, dorsomedial nucleus of the hypothalamus; DMV, dorsal motor nucleus of the vagus; IML, intermediolateral column; INS, insular cortex; LC, locus ceruleus; LH, lateral hypothalamus, MPO, medial preoptic area; NTS, nucleus tractus solitarius; OVLT, organum vasculosum of the lamina terminalis; PFC, prefrontal cortex; PVN, paraventricular hypothalamus; RCA, retrochiasmatic area; SCN, suprachiasmatic nucleus; SFO, subfornical organ; VMH, ventromedial hypothalamus; ZI, zona incerta (reproduced with permission from ref. 110)

intrapancreatic ganglia, and terminate close to the islet endocrine cells (Figure 8). The intrapancreatic ganglia are in turn controlled by preganglionic fibers which originate in the DMV, traverse the vagus as part of the bulbar outflow tract, and enter the pancreas along the cranial and caudal pancreaticoduodenal arteries. The adrenergic nerves innervating the islets are also post-

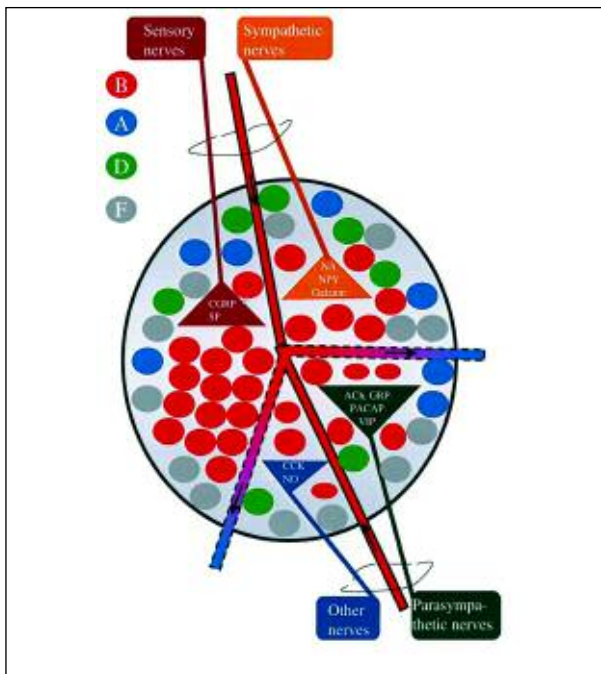


Figure 8. Schematic view of the innervation of a pancreatic islet with the main branches of the autonomic nerves (parasympathetic nerves, sympathetic nerves, sensory nerves and other nerves) with their respective neurotransmitters. The four main types of islet endocrine cells are also illustrated with beta cells (B) forming the central islet portion, whereas an islet mantle zone harbors alpha cells (A cells, glucagon cells), delta cells (D cells, somatostatin cells) and F cells (pancreatic polypeptide (PP) cells). Afferent vessels (red) and fenestrated efferent vessels (red to blue) are also illustrated (arrows indicate blood flow direction). Ach = acetylcholine, NO = nitric oxide (reproduced with permission from ref. 122)

ganglionic and their nerve cell bodies are located in either the celiac ganglion or the paravertebral sympathetic ganglia. The postganglionic fibers then pass from the ganglia within the mixed autonomic nerves and enter the pancreas along its blood vessels.

It has been demonstrated that stimulation of the vagus (parasympathetic) and splanchnic (sympathetic) nerves can alter insulin and glucagon secretion. Stimulation of the vagus nerve increases insulin release by predominantly muscarinic mechanisms, and vagal non-muscarinic (possibly peptidergic) mechanisms may mediate changes in glucagon secretion. Stimulation of α -adrenergic receptors inhibits insulin secretion and β -adrenergic stimulation increases insulin release, whereas both α - and β -adrenergic stimulation

increases the secretion of glucagon (112-122). However, despite the wealth of literature dealing with the effects of autonomic neural stimulation on islet hormone secretion, it is intriguing to note that complete denervation of the canine pancreas has no effect on the *in vivo* insulin and glucagon responses to exercise (123). These findings indicate that although *in vitro* experimental stimulation of the parasympathetic and sympathetic nervous input to the pancreas does cause the aforementioned changes in insulin and glucagon secretion, the autonomic innervation does not appear essential for the *in vivo* generation of islet cell responses that occur during moderate exercise. Moreover, these islet cell responses to exercise are unlikely to be due to muscle-derived IL-6 since this cytokine has been shown to stimulate, rather than inhibit, β -cell secretion of insulin (124). To reconcile these observations, we propose that:

- *The acute changes in insulin and glucagon secretion during moderate exercise are hormonally mediated by an acute release of Musculin from exercising muscle.*
- *The autonomic nervous system may become increasingly important as a regulator of islet hormone secretion as exercise intensity increases.*

Studies of the acute effects of endurance exercise on the autonomic nervous system in man suggest that inhibition of cardiac vagal activity occurs very early after the onset of exercise, whereas sympathetic activation occurs later and becomes more pronounced as exercise intensity increases (125, 126). However, as judged by measurements of plasma catecholamines and whole-body norepinephrine (NE) spillover into plasma, prolonged endurance training actually reduces sympathetic nervous system activity (127-134). This reduction in sympathetic nervous system activity is likely to be a tissue-specific response and has been shown to occur in the kidney, but not in the heart (132). To our knowledge, no studies have directly determined the long-term effect of endurance exercise in animals or man on NE spillover into plasma from the pancreas but, based on the available evidence, it seems reasonable to suggest that it would be either reduced or unaltered, but not increased.

As noted previously, a number of studies have shown that long-term endurance exercise lowers plasma insulin concentrations. Like most, if not all, en-

doctrine tissues, the β -cell secretes insulin in a pulsatile fashion and Engdahl et al. (90) have shown that long-term endurance exercise reduces the mass of insulin secreted per burst, the burst height and insulin production rate, but has no effect on the interpulse interval or burst half-duration (Figure 9). If one was to ascribe these alterations in insulin secretion solely to an alteration in sympathetic nervous system activity, one would have to postulate that long-term endurance exercise had increased pancreatic sympathetic nervous system activity. However, since as noted above, there is currently no available evidence to suggest that long-term endurance activity increases NE spillover into plasma from any organ, this explanation would appear unlikely. Rather, we postulate that:

- Long-term endurance exercise may upregulate Musculin synthesis and secretion from skeletal myocytes.
- The resultant increase in serum Musculin concentrations may augment the tonic inhibitory regulation of the β -cell, thereby reducing the steady-state level of insulin in plasma.

Insulin secretion in states of decreased muscle insulin sensitivity

The concept that resistance to the action of insulin may be pathophysiologically important in some patients with diabetes was first enunciated by Himsworth in the 1930s (135-138). The development of an insulin bioassay by Bornstein and Lawrence (139) and the ground-breaking insulin radioimmunoassay by Yalow and Berson (140) conclusively demonstrated that hyperinsulinemia was present in type 2 diabetic patients, and this finding was confirmed by others. Although the meaning of these observations was debated for some years, the measurement of insulin-mediated glucose disposal during a continuous infusion of insulin-glucose-epinephrine-propranolol (141), and subsequently by the hyperinsulinemic-euglycemic clamp technique (142), conclusively demonstrated the defective ability of insulin to increase tissue utilization of glucose in most patients with type 2 diabetes. It is now widely appreciated that insulin resistance may an-

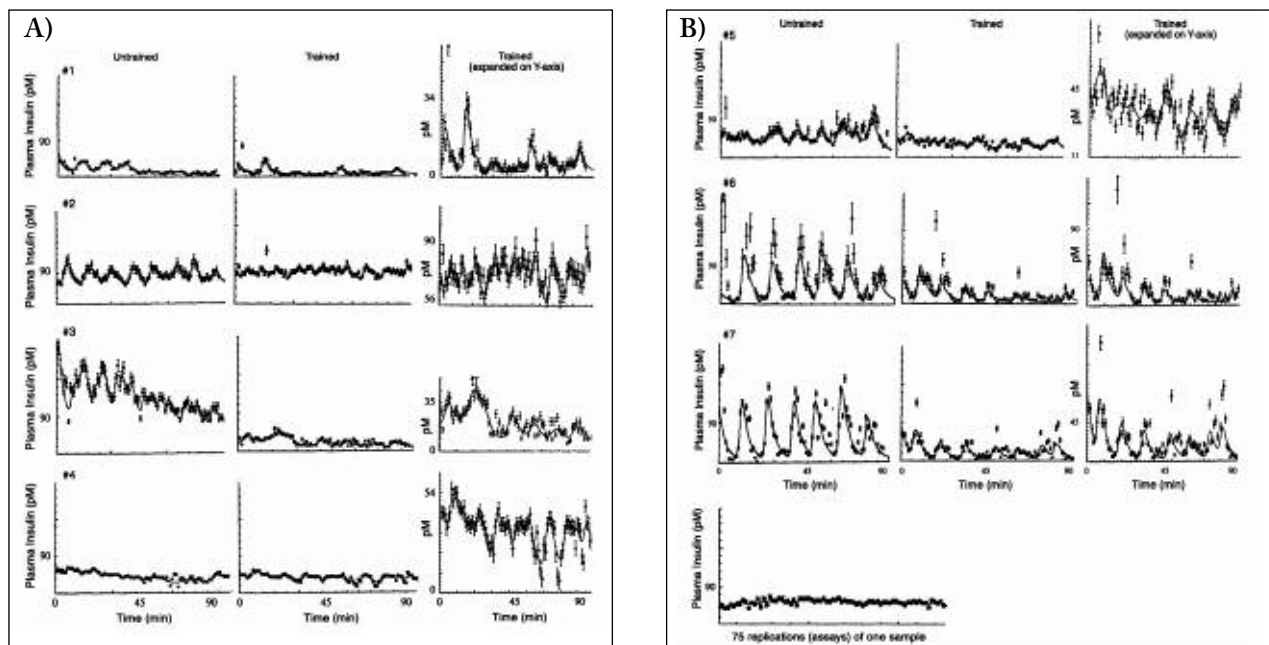


Figure 9A and 9B. Effect of long-term endurance training on pulsatile insulin secretion. Individual pulse profiles for 7 untrained and 7 trained men. Solid lines, best fit from deconvolution analysis. Nos. at upper left, subject no. for each pair of subjects whose data are shown side by side were assayed during the same assay. For purposes of comparison, right column shows same data as middle column; however, y-axis has been expanded so that pulses can be visualized. Note: ranges for insulin concentrations on y-axis are not the same as other panels. Single graph at bottom of panel B represents sample of blood that was assayed in duplicate 75 times during 1 assay (reproduced with permission from ref. 90)

tedate the development of diabetes by many years (144-147), and that the hyperinsulinemia *per se* may contribute to the development of dyslipidemia and hypertension, which in turn increases an individual's risk for the development of cardiovascular disease. In 1988, Reaven coined the term Syndrome X (143) to describe the association of insulin resistance, hyperinsulinemia, varying degrees of glucose intolerance and dyslipidemia, although with the inclusion of numerous other metabolic abnormalities, this nomenclature has given way to the term 'The Insulin Resistance Syndrome' (148). A schematic representation of the mechanisms leading from muscle insulin resistance to Impaired Glucose Tolerance and frank Diabetes is shown in Figure 10.

The molecular basis of insulin resistance in insulin target tissues has been the focus of intense research during the last decade (149-170). Studies by Hotamisligil and coworkers have demonstrated that obesity activates cellular stress signaling and inflammatory pathways in the adipocyte and it now appears that the

stress signals originate in the endoplasmic reticulum (ER) (151-153, 166, 167, 170). The ER is involved in the secretion and processing of membrane proteins, but biological insults such as infection, hypoxia, or exposure to excess lipids can disrupt ER function, causing unfolded or misfolded proteins to accumulate in the ER lumen. To compensate for this stress, the ER activates a transcriptional program termed the 'unfolded protein response' which slows protein synthesis and promotes protein degradation (Figure 11). This sequence of events impairs insulin receptor signaling in the adipocyte (and liver) of obese animals and results in insulin resistance in these tissues. However, ER stress seems not to be present in the skeletal muscle of obese animals (167), suggesting that alternative mechanisms must underly insulin resistance in this site.

Since skeletal muscle accounts for up to 80% of the total daily insulin-mediated glucose disposal in man, insulin resistance in skeletal muscle must contribute significantly to the insulin resistance defect in

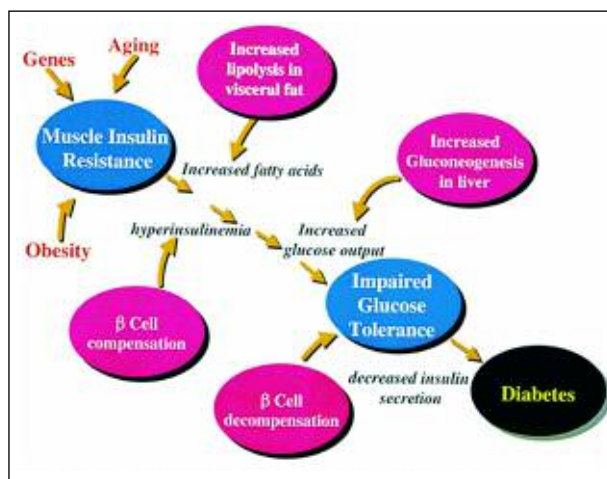


Figure 10. Metabolic Staging of Type 2 Diabetes. Type 2 Diabetes is characterized by a progressive decrease in insulin action, followed by an inability of the β cell to compensate for insulin resistance. Insulin resistance is the first lesion, due to interactions among genes, aging, and metabolic changes produced by obesity. Insulin resistance in visceral fat leads to increased fatty acid production, which exacerbates insulin resistance in liver and muscle. The β cell compensates for insulin resistance by secreting more insulin. Ultimately, the β cell can no longer compensate, leading to impaired glucose tolerance and diabetes (reproduced with permission from ref. 161)

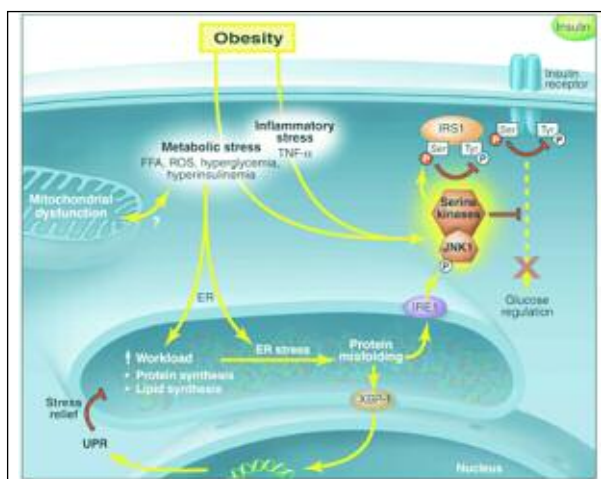


Figure 11. No stress relief for the Endoplasmic Reticulum (ER). The metabolic and inflammatory stresses of obesity disrupt the smooth operation of the ER and cause protein misfolding. The ER attempts to cope with stress by activating XBP-1, a transcriptional regulator of the unfolded protein response (UPR). If these responses fail to restore homeostasis, stress-induced IRE1 activates JNK1, a serine kinase that opposes insulin action. Impaired insulin signaling might serve to alleviate intracellular stress, but it does so at the expense of systemic glucose regulation. FFA, free fatty acids; ROS, reactive oxygen species (reproduced with permission from ref. 166)

most, if not all, patients with diabetes (142). Although insulin resistance can be selectively induced in skeletal muscle of laboratory animals by deletion of those genes coding for the GLUT4 glucose transporter and the insulin receptor (Figure 12), as well as PPAR γ and caveolin-3 (154, 160, 162-164, 168), the studies of Shulman and coworkers point towards mitochondrial dysfunction as being perhaps the most important me-

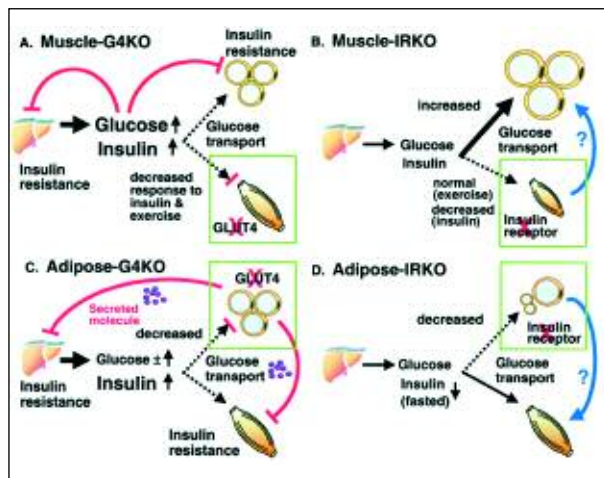


Figure 12. Changes in glucose homeostasis and adiposity with muscle-specific or adipose-specific ablation of GLUT4 or IR. *A*, muscle-G4KO mouse. Ablation of GLUT4 from muscle (*green box*) decreases both insulin- and exercise-induced glucose uptake in muscle resulting in hyperglycemia, hyperinsulinemia, and secondary insulin resistance in the liver and adipose tissue. Insulin resistance in the liver and adipose tissue may be caused, at least partly, by glucose toxicity (*red curves*). *B*, muscle-IRKO (or MIRKO) mouse. Ablation of IR in muscle (*green box*) decreases muscle mass but does not change plasma glucose or insulin levels or glucose tolerance. Contraction-stimulated glucose uptake remains normal. Increased glucose uptake into adipose tissue increases adipose mass, serum triglycerides, and free fatty acids. Whether muscle releases a factor that directly acts on adipose tissue is unknown (*blue curve*). *C*, adipose-G4KO mouse. Ablation of GLUT4 in adipose tissue (*green box*) does not alter adipose mass, but results in insulin resistance in liver and muscle and systemic hyperinsulinemia. This is most likely due to altered secretion of an unknown molecule(s) from adipose tissue (*red curves*). Blood glucose is increased in some of the adipose-G4KO mice (*symbol ±*). *D*, adipose-IRKO (or FIRKO) mouse. In contrast to the adipose-G4KO mouse, ablation of IR in adipose tissue (*green box*) decreases adipose mass, lowers fasting insulin levels, and may increase energy expenditure. This may, in part, be driven by changes in adipocyte-secreted molecules (*blue arrow*). *Red*, insulin resistance; *blue*, insulin action or sensitivity (reproduced with permission from ref. 162)

chanism underlying common forms of skeletal muscle insulin resistance (150, 155, 156, 158, 165, 169, Figure 13). With the use of ^{13}C NMR spectroscopy to measure the rate of $[1-^{13}\text{C}]$ glucose incorporation into muscle glycogen, these investigators have demonstrated that muscle glycogen synthesis is the major pathway for glucose metabolism in both normal and diabetic individuals during steady-state hyperglycemic, hyperinsulinemic conditions and that muscle glycogen synthesis is $\sim 50\%$ lower in diabetics. Furthermore, the data suggest that insulin-stimulated glucose transport, rather than defective hexokinase II activity, is the rate-limiting step for muscle glycogen synthesis and is reduced in diabetic subjects.

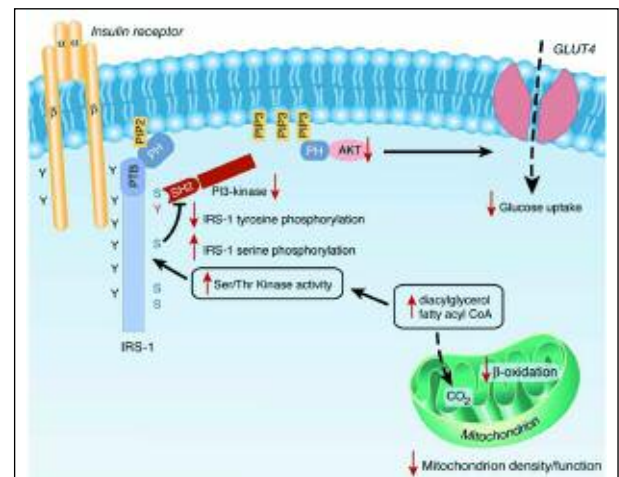


Figure 13. Potential mechanism by which mitochondrial dysfunction induces insulin resistance in skeletal muscle. In the depicted model, a decrease in mitochondrial fatty acid oxidation, caused by mitochondrial dysfunction and/or reduced mitochondrial content, produces increased levels of intracellular fatty acyl CoA and diacylglycerol. These molecules activate novel protein kinase C, which in turn activates a serine kinase cascade [possibly involving inhibitor of nuclear factor κB kinase (IKK) and c-jun N-terminal kinase-1], leading to increased serine phosphorylation (pS) of insulin receptor substrate-1 (IRS-1). Increased serine phosphorylation of IRS-1 on critical sites (e.g. IRS-1 Ser 307) blocks IRS-1 tyrosine (Y) phosphorylation by the insulin receptor, which in turn inhibits the activity of phosphatidylinositol 3-kinase (PI 3-kinase). This inhibition results in suppression of insulin-stimulated glucose transport, the process by which glucose is removed from the blood. PIP3 indicates phosphatidylinositol 3,4,5-trisphosphate; PTB, phosphotyrosine binding domain; PH, pleckstrin homology domain; SH2, src homology domain (reproduced with permission from ref. 169)

The finding of an inverse relationship between plasma free fatty acid concentrations, intramuscular triglyceride content and insulin sensitivity provides evidence for a causal relationship between altered fatty acid metabolism and insulin resistance in diabetes (156, 157). In the 1960s, Randle et al. (149) suggested that an increased supply of fatty acids competed with glucose for oxidation to increase intracellular levels of acetyl CoA and citrate. This was thought to lead to an inhibition of pyruvate dehydrogenase and phosphofructokinase which was thought to then increase intracellular glucose and glucose-6-phosphate concentrations, thereby resulting in reduced insulin-stimulated glucose uptake. This idea has essentially been refuted and it is now thought that fatty acids alter cellular activation of the protein kinase C isozymes, epsilon and theta, and cause serine phosphorylation of IRS-1, thereby abolishing insulin-stimulated IRS-1-associated PI-3 kinase activity and reducing glucose transport. Recently, insulin resistance in healthy, non-diabetic elderly individuals has also been shown to be due to increased intramyocellular fatty acid metabolites causing the aforementioned cascade of events (165). The findings may be due to an age-related reduction in mitochondrial oxidation and phosphorylation capacity, due either to a reduction in number and/or function of the mitochondria with advancing age.

Although, as stated above, the net effect of these changes in the intracellular milieu of the insulin-resistant adipocyte and myocyte is to reduce insulin-mediated glucose transport, tissue-specific differences in glucose transporter protein abundance in these cells have been reported (171-175). For example, the reduction in glucose uptake into the adipocyte is due in large part to decreased abundance of the GLUT4 protein consequent upon a reduction in GLUT4 mRNA expression in that tissue. In contrast, GLUT4 abundance and mRNA expression are not significantly altered in the myocyte, and the defect in glucose transport into that tissue may be largely due to defective trafficking of the GLUT4 protein from the cytosol to the cell membrane. This defect may be a consequence of alterations in levels (and/or function) of the vesicle transport proteins cellubrevin, VAMP-2, and syntaxin-4. Such alterations have been found in the ske-

letal muscle of the Zucker diabetic fatty rat, a model of type 2 diabetes (49). Furthermore, the correction of these abnormal levels by the restoration of normoinsulinemia, and their absence in the streptozotocin-diabetic rat model of type 1 diabetes, suggests that hyperinsulinemia *per se*, rather than hyperglycemia, is responsible for these changes. From these observations, we postulate that:

- *Perturbations of the vesicular transport proteins in the insulin-resistant myocyte could contribute to defective transport to the cell surface of other intracellular myocyte proteins.*
- *These perturbations could provide a structural basis for the suggestion that Musculin secretion may be defective in insulin resistant states.*

The regulation of the pancreatic β -cell mass

Although it was previously thought that the β -cell mass was static, it is now believed to be dynamic and, at any moment in time, total β -cell number is the result of a balance between neogenesis, replication, and apoptosis (176-180). For example, the first two weeks of the neonatal rat's life is characterized by marked β -cell mitosis and neogenesis which is followed by a wave of β -cell apoptosis from postnatal days 13 to 24. During the first three weeks of life, β -cell mass and body weight are not tightly correlated and, during this time, large fluctuations in the concentration of β -cell peptides have been observed (181-184). However, from day 24 onwards, β -cell mass and body weight are highly correlated, although the factors responsible for this tight regulation are unclear. The demonstration in the mouse that pre-existing, terminally differentiated β -cells, rather than pluripotent stem cells, are the major source of new β -cells formed during adult life and following partial pancreatectomy (185,186), implies a role for cell cycle regulation in this process.

The mammalian G1 cyclins and their associated kinases (cdk) integrate extracellular mitogenic signals and regulate the cell division cycle (187-192, Figure 14). The three D-type cyclins (D1, D2, and D3) bind to and regulate one of two cdk subunits, cdk4 and cdk6, as well as the E-type cyclins (E1 and E2) which, in similar fashion, govern the activity of a single

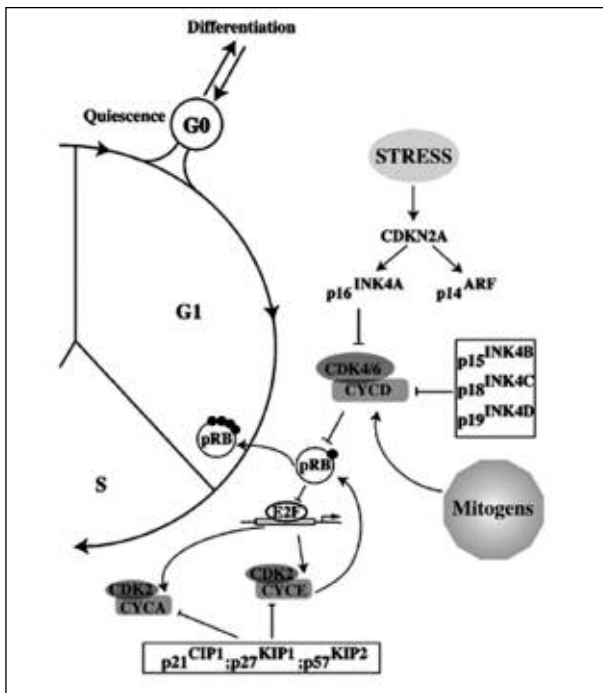


Figure 14. G1-to-S cell cycle control. Production of D-type cyclins and activation of cdk 4/5 in response to mitogens results in phosphorylation and inactivation of pRB (and family members) with consequent derepression of E2F-dependent transcription. This results in cyclin E and A synthesis, activation of cdk2 and further pRB phosphorylation. The activity of cdk 4/6 is opposed by p16INK4a, produced in response to stress, or by other members of the INK4 family, produced in response to differentiation signals. In a conceptually similar manner, the activity of cdk2 is opposed by members of the CIP/KIP family of inhibitors, also produced in response to stress and differentiation signals. In contrast to INK4 proteins, CIP/KIP inhibitors can act as assembly factors for cycD/cdk4(6) complexes, and can be titrated away from cdk2 by these D cyclin-containing complexes (reproduced with permission from ref. 192)

catalytic subunit, cdk2. Mitogen-induced signal transduction pathways promote the activation of cyclin D-cdk complexes which then inactivate two classes of cell cycle inhibitors. The cdk2 also phosphorylates Retinoblastoma (Rb) protein family members (Rb, p107, and p130) thus inactivating their transcriptional co-repressor activities. This process controls an E2F-dependent transcriptional program that activates a battery of genes whose products are required for DNA replication and metabolism.

The relevance of this information is highlighted by the demonstration that cyclin D2 is expressed in

the nuclei of a subset of β -cells and that deletion of the cyclin D2 gene has revealed an essential role for cyclin D2 in the normal postnatal development of the β -cell mass. In addition, the β -cells of *cyclin D2*^{-/-} mice are unable to upregulate expression of the remaining D-cyclins and eventually develop glucose intolerance. However, in all other respects, *cyclin D2*^{-/-} mice are phenotypically indistinguishable from control animals (186). In addition, *Cdk4*^{-/-} mice also display defective β -cell proliferation and develop insulin-deficient diabetes, indicating that Cdk4 acts in partnership with cyclin D2 to regulate cell cycle progression in β -cells (194). However, in contrast to *cyclin D2*^{-/-} mice, *Cdk4*^{-/-} animals are smaller than normal and infertile, indicating that Cdk4 may play a more general role in development.

In addition, studies in rodents and man have demonstrated that the β -cell mass increases in insulin resistant states, although the magnitude of the effect is far greater in the rodent. The generation of double heterozygous *IR/IRS-1*^{-/-} mice causes severe muscle insulin resistance, a 2.6-fold rise in plasma insulin concentrations, a 2-30-fold (mean: 10-fold) increase in β -cell mass, and eventual diabetes (195). Moreover, an analysis of human pancreata obtained at autopsy has shown that the relative β -cell volume is increased by ~50% in obese versus lean nondiabetic individuals, an effect that is due to increased islet neogenesis, since the frequency of apoptosis did not differ significantly between the two groups (196). With these findings in mind, we postulate that:

- *A decline in Musculin secretion that occurs as a consequence of decreased skeletal muscle insulin sensitivity could stimulate β -cell neogenesis by upregulating β -cell cyclin D2 and/or Cdk4 gene expression (Figure 15).*

In this regard, it is noteworthy that a precedent for the hormonal regulation of Cyclin gene expression has been established by the studies of Sicinski et al (197) which have shown ovarian Cyclin D2 to be a follicle-stimulating hormone (FSH) -responsive gene. The finding that β -cell hyperplasia occurs in *IR/IRS*^{-/-} mice before the development of hyperglycemia has led to the suggestion that a factor(s) in addition to glucose, is responsible for the expansion of the β -cell mass in the mouse (195), and the studies of Flier et al (198)

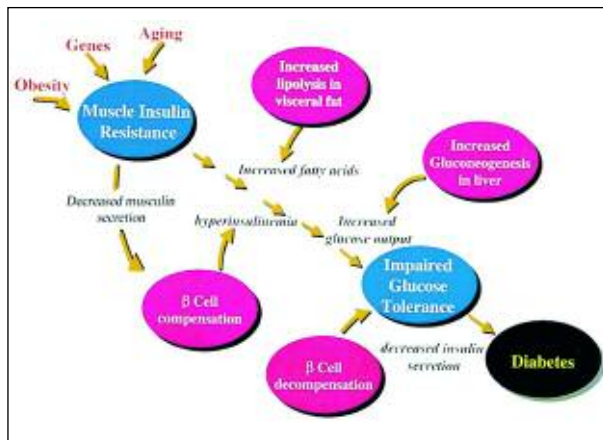


Figure 15. Metabolic Staging of Type 2 Diabetes. This figure is a modification of Figure 10 and proposes that the hyperinsulinemia that occurs as a consequence of skeletal muscle insulin resistance is due to a reduction in a tonic inhibitory regulation of insulin secretion and synthesis by the pancreatic β -cell. The inhibitory regulation is postulated to be due to secretion of a putative skeletal muscle hormone termed *Musculin* and decreased skeletal muscle insulin sensitivity (or increased skeletal muscle insulin resistance) is proposed to result in reduced secretion of the hormone

also lend support to this hypothesis. These authors transplanted wild-type (WT) islets under the kidney capsule of *IR/IRS-1^{-/-}* and *ob/ob* mice and in both cases, the β -cell volume increased significantly, due to increased β -cell mitosis. In contrast, islets from *IR/IRS-1^{-/-}* mice, when transplanted into WT recipients, displayed a reduced mitotic index. However, the authors were unable to specify the nature of this factor or ascertain its source. In previous studies, Kahn and colleagues (154, 199) have reported the effects of the conditional inactivation in mice of the insulin receptor in liver (LIRKO) or muscle (MIRKO), and noted marked islet hyperplasia in the LIRKO animals, but normal sized islets in the MIRKO mice. The authors therefore suggested that the findings did not lend support to the notion that this mitogenic factor was of skeletal myocyte origin, although whether the perturbation of the intracellular milieu that follows a complete loss of muscle insulin receptor function is identical to that which results from mitochondrial dysfunction causing the common form of human insulin resistance remains to be determined.

Evidence that musculin may act on the hypothalamus to coordinate the neuroendocrine and appetite response to exercise

It has long been appreciated that acute exercise causes a neuroendocrine response that includes reproducible and robust increases in serum GH, adrenocorticotropic (ACTH), and prolactin and less marked increments in serum thyrotropin (TSH), FSH, oestradiol and progesterone (in women), and testosterone in men (200–245). In view of these findings and in the interest of space, only the possible mechanisms underlying the GH, ACTH, and prolactin responses to exercise are discussed below. Physical exercise also produces a change in appetite characterized by short-term anorexia (246–254). In order to facilitate an understanding of the possible mechanisms by which these adaptations are mediated, we firstly provide a brief outline of the hypothalamic regulation of anterior pituitary hormone secretion and of the anatomy and connections of the hypothalamic nuclei that are likely to be involved.

The hypothalamic regulation of anterior pituitary hormone secretion

GH

The two main hypothalamic neuropeptides that regulate GH secretion are GH-Releasing Factor (GRF) and Somatostatin (255, 256, Figure 16). A third important stimulatory input to GH secretion is provided by the peptide Ghrelin that is released from the stomach (257), but Ghrelin will not be further discussed in this manuscript since its plasma concentrations are unaffected by exercise (258). GH stimulates the hepatic production of Insulin-Like Growth Factor-1 (IGF-1) which then exerts a negative feedback on GH by stimulating the hypothalamic release of somatostatin and by decreasing GH secretion and gene expression in the anterior pituitary (259–262).

Human GRF is a 44-residue neuropeptide that was originally isolated from a human pancreatic tumor that caused acromegaly (263, 264). GRF is synthesized in the arcuate nucleus (ARC) of the hypothalamus and GRF axons abut the long portal vessels from whe-

re the peptide is secreted in a pulsatile manner into the hypophysial-portal circulation (265, 266). GRF binds to a specific receptor on the somatotrophic cell membrane and results in an increase in cyclic AMP and Ca^{2+} concentrations, an activation of protein kinase A, and a stimulation of GH secretion and GH gene expression (267-271).

Somatostatin-14 (SRIF, SS-14) is present in several hypothalamic areas including the anterior periventricular area and the ARC, but it is only the anterior periventricular area which sends axons to the external zone of the median eminence from where the peptide enters the hypophysial-portal circulation (266, 272-274). SS-14 acts on specific receptor(s) on the somatotrope cell membrane and reduces Ca^{2+} concentrations, decreases GH secretion and suppresses the GRF-induced increase in GH gene transcription. However, SS-14 does not seem to exert an appreciable effect on basal GH gene transcription (275-278).

ACTH

It has been traditionally thought that the hypothalamus only exerts a stimulatory influence upon the secretion and synthesis of ACTH and that this is mediated by the neuropeptides corticotropin-releasing factor (CRF), arginine vasopressin (AVP), and oxytocin (OT) which are secreted into the hypophysial-portal circulation (279-284, Figure 17). ACTH then stimulates the adrenocortical secretion of cortisol which exerts a negative feedback effect on ACTH release by acting on hypothalamic and extrahypothalamic brain sites as well as on the anterior pituitary corticotropes.

However, studies of the hypothalamic-pituitary-adrenal (HPA) axis in animals in which the pituitary has been surgically disconnected from the hypothalamus have suggested that the hypothalamus may exert both stimulatory and inhibitory regulation over ACTH secretion and proopiomelanocortin (POMC) biosynthesis, and the inhibitory regulation has been postulated to be mediated by a currently unidentified substance termed Corticotropin Release-Inhibitory Factor (282, Figure 18).

CRF is a 41-residue peptide that is the most potent ACTH secretagogue in the rat although its abi-

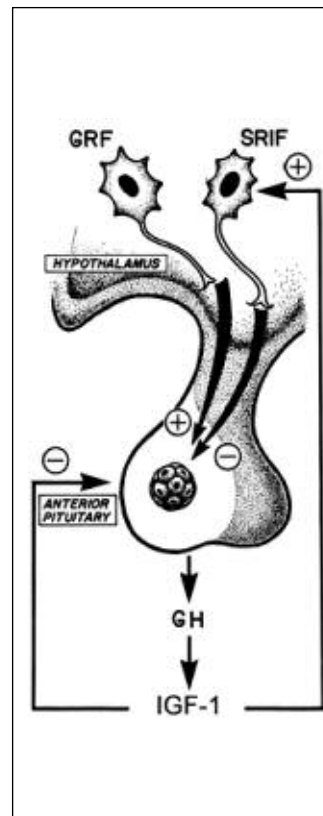


Figure 16. A schematic representation of the current model of the regulation of Growth Hormone secretion. The hypothalamus both stimulates and inhibits Growth Hormone (GH) secretion by secreting Growth Hormone-Releasing Factor (GRF) and Somatostatin (SRIF) into the hypophysial-portal circulation. GH then stimulates the hepatic production of Insulin-Like Growth Factor-1 (IGF-1) which exerts a stimulatory effect on hypothalamic SRIF release and an inhibitory effect at the level of the anterior pituitary to decrease GH release and biosynthesis. Although not shown in the diagram, GH release is also stimulated by the stomach-derived peptide Ghrelin which acts on a specific anterior pituitary receptor

lity to stimulate ACTH secretion is potentiated several-fold by agonists such as AVP, OT, angiotensin II, NE and EPI (285, 286). However, CRF may not be the most potent ACTH secretagogue in all species as

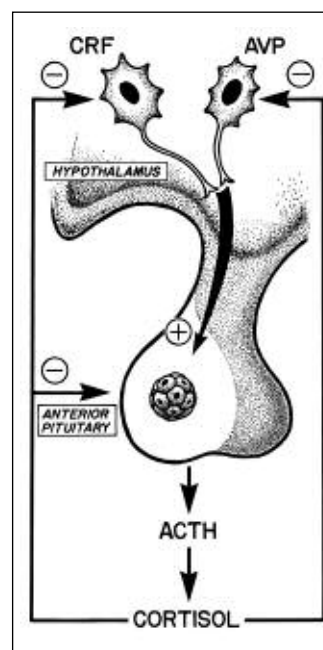


Figure 17. A schematic representation of the current model by which the hypothalamus is thought to regulate ACTH secretion. This model proposes that the hypothalamus only stimulates ACTH secretion by secreting neuropeptides such as CRF and AVP into the hypophysial-portal circulation. ACTH then stimulates the adrenocortical production of cortisol, which then restrains the secretion of ACTH by exerting negative feedback effects on the anterior pituitary, hypothalamus, and various extrahypothalamic brain sites (reproduced with permission from ref. 282)

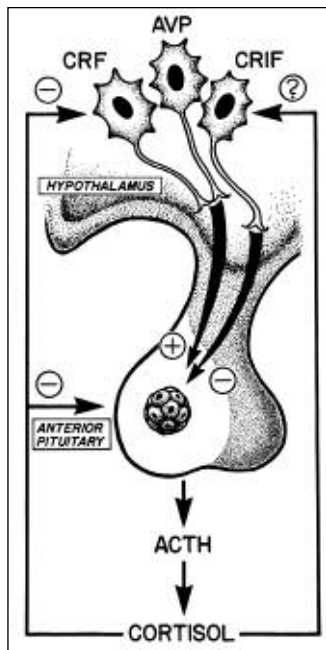


Figure 18. A postulated bidirectional model of the way the hypothalamus may regulate ACTH secretion. This is a model that postulates that the hypothalamus may both stimulate and inhibit ACTH secretion. Moreover, it suggests that the hypothalamic inhibition of ACTH release is mediated by the secretion of a single CRIF. However, it is possible that several substances could cooperate to mediate the inhibition by acting in an analogous fashion to the stimulatory interaction of CRF and AVP (reproduced with permission from ref. 282)

it appears to be equipotent with AVP in the bovine species and in the ovine species, AVP appears to be even more potent than CRF (287-289). In the rat, CRF is the only hypothalamic neuropeptide known to increase POMC biosynthesis, and none of the aforementioned ACTH secretagogues are able to potentiate this effect of the peptide (290-294). CRF exerts its effects on the anterior pituitary by binding to a specific adenylate cyclase-linked receptor. The binding of the hormone to the corticotropic cell membrane results in increased intracellular concentrations of cAMP, an increased influx of extracellular Ca^{2+} , and an activation of protein kinase A and phosphorylation of a number of intracellular proteins (295-302).

AVP is also secreted into the hypophysial-portal circulation and acts on the anterior pituitary to stimulate ACTH release. AVP is a weak ACTH secretagogue in the rat and in man, although as noted above, this order of potency may not pertain in all species. AVP binds to the V1b receptor on the corticotropic cell which is coupled to the PI signaling pathway and therefore hormone binding increases the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (303-319, DG). The DG is required for the activation of protein kinase C which phosphorylates a number of intracellular substrates. The IP_3 causes the liberation of Ca^{2+} from intracellular stores and, together with the

influx of extracellular Ca^{2+} , causes the rise in Ca^{2+}_i that is required to mediate ACTH release.

Prolactin

Prolactin is synthesized by the lactotropes of the anterior pituitary gland which possess a high intrinsic hormonal secretory activity and are under tonic hypothalamic inhibitory control mediated by dopamine (DA) in the hypophysial-portal circulation (320-324, Figure 19). Therefore, the ability of a given stimulus to reduce the tonic inhibitory effects of DA on the lactotrope is a cardinal, but not the sole, mechanism that increases prolactin secretion. The anterior pituitary lactotropic D2 DA receptor is coupled to $G_{i-3}\alpha$ and DA binding causes inhibition of the adenylate cyclase and inositol phosphate metabolism, inhibition of Ca^{2+} channels, and excitation of voltage-sensitive K^+ channels (325-328). However, DA withdrawal also leads to activation of protein kinase A which causes phosphorylation of intracellular substrates including Ca^{2+} channels, thereby increasing the probability of Ca^{2+} channels being open, and promoting the influx of extracellular Ca^{2+} . In this man-

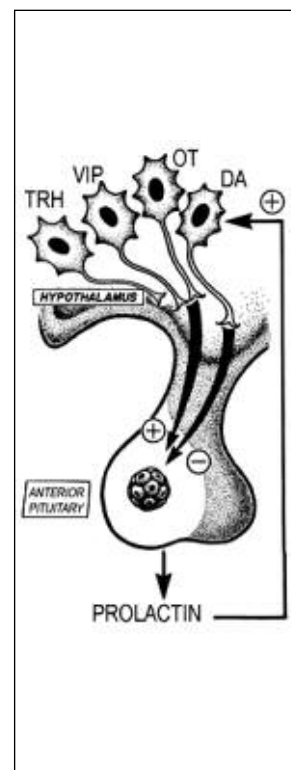


Figure 19. A schematic representation of the regulation of prolactin secretion. The hypothalamic Tuberoinfundibular Dopamine neurons (TIDA) secrete Dopamine (DA) into the hypophysial-portal circulation which exerts a dominant inhibitory influence on the anterior pituitary lactotrophs to restrain prolactin secretion and synthesis. The activity of the TIDA neurons are in turn subjected to both positive and negative regulation by numerous hypothalamic amines and neuropeptides. The hypothalamus also stimulates prolactin secretion by secreting neuropeptides such as Thyrotropin-Releasing Hormone (TRH), Vasoactive-Intestinal Peptide (VIP) and Oxytocin (OT) into hypophysial-portal blood. Prolactin in turn restrains its own secretion by means of an ultra-short loop feedback effect that involves stimulation of TIDA neuronal activity

ner, DA potentiates the prolactin-releasing activity of secretagogues such as Thyrotropin-Releasing Hormone (TRH) which predominantly act by the protein kinase C pathway (329, 330). In contrast to GH and ACTH which stimulate the synthesis of additional hormones in peripheral target organs that in turn restrain their secretion by negative feedback effects on the pituitary and hypothalamus, prolactin regulates its own secretion by a positive short-loop feedback mechanism that involves activation of those tuberoinfundibular neurons that project to the external zone of the median eminence (331, 332). As alluded to above, additional regulatory inputs to prolactin secretion exist in the form of hypothalamic releasing factors, the best studied of which are TRH, vasoactive intestinal peptide (VIP) and OT.

TRH was initially isolated as a hypophysiotropic factor capable of stimulating TSH secretion from anterior pituitary cells but was subsequently found to stimulate prolactin release both *in vitro* and *in vivo*. TRH is secreted into the hypophysial-portal circulation and the TRH receptor is located in the anterior pituitary, specifically on lactotropes and thyrotropes (333-340). The binding of TRH to its receptor activates phospholipase C which initiates a cascade of intracellular signaling events that are similar, or identical, to those produced by the aforementioned binding of AVP to the anterior pituitary V1b receptor (341, 342). As noted above, several studies indicate that transient DA antagonism, or withdrawal, may augment TRH-stimulated prolactin secretion, although whether tuberoinfundibular neurons are ever truly quiescent under physiological conditions is open to conjecture.

VIP was initially isolated from porcine small intestine but VIP-immunoreactive(-ir) perikarya are also found in the hypothalamic paraventricular nucleus (PVH). VIP-ir is found in nerve terminals in the external zone of the median eminence from where the peptide is secreted into hypophysial-portal blood (343-346). VIP binds to a specific receptor on the anterior pituitary and causes an activation of the adenylate cyclase, a rise in Ca^{2+}_i and phosphorylation of a set of intracellular proteins that are distinct from those phosphorylated by TRH (347-351). Studies employing the technique of VIP immunoneutralization with specific VIP antisera have shown that VIP is entirely responsi-

ble for the ether stress-induced rise in prolactin. Moreover, VIP is required for the acute prolactin response to suckling and is one of the prolactin releasing factors required for maintenance of the hyperprolactinemia in continuously suckling animals (352).

OT synthesized in the parvocellular part of the PVH as well as the periventricular nucleus (Pv) reaches the anterior pituitary by secretion into the long portal vessels. In addition, the OT synthesized in the magnocellular divisions of the PVH and the supraoptic nucleus (SON) is transported by axoplasmic flow to the posterior pituitary from where it may reach the adenohypophysis by means of the short portal vessels which connect the neural lobe with the inner zone of the anterior lobe (353-355). The oxytocinergic neurons in turn receive inhibitory inputs from VIP neurons originating in the suprachiasmatic nucleus (SCN). OT binds to a specific receptor on lactotropes and causes a dose-related increase in prolactin release *in vitro* and Ca^{2+}_i concentrations in these cells (356, 357). Studies employing specific OT antisera have shown that endogenous OT is likely to be one factor that mediates the prolactin response to suckling and studies with OT antagonists indicate that OT is required for the prolactin rise on the afternoon of proestrus, but is not involved in the prolactin response to ether stress (358, 359). Stimulation of the uterine cervix during mating also causes unique nocturnal and diurnal surges of prolactin secretion and a role for OT in mediating this response is demonstrated by the abolition of the afternoon rise in prolactin and OT by injection of VIP antisense oligonucleotides into the SCN (357).

Taken together, these findings indicate that multiple hypothalamic factors regulate the release of prolactin and that the various components of this system can be activated in a stimulus-specific manner.

The hypothalamic nuclei involved in the regulation of anterior pituitary hormone secretion and appetite

PVH

The PVH lies on either side of the third ventricle and can be divided into at least eight clearly defined subdivisions (Figure 20).

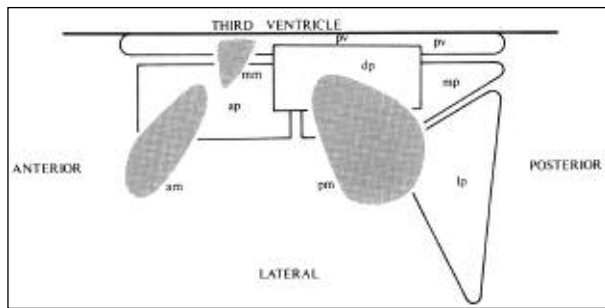


Figure 20. A schematic diagram to show the major cell groups of the paraventricular nucleus of the hypothalamus in the rat, as viewed from above. The three parts of the magnocellular division are shown in *stipple* and are embedded in the parvocellular division, which consists of five parts. The abbreviations are as follows: am, anterior magnocellular; ap, anterior parvocellular; dp, dorsal parvocellular; lp, lateral parvocellular; mm, medial magnocellular; mp, medial parvocellular; pm, posterior magnocellular; pv, periventricular (reproduced with permission from ref. 282)

Three of these are magnocellular (anterior, medial, posterior) that project to the posterior pituitary and five are parvocellular (periventricular, anterior, medial, dorsal, lateral) which project to the external zone of the median eminence and are also interrelated with autonomic cell groups in the brain stem and spinal cord via bidirectional pathways (360–362). The PVH is also connected with a number of brain regions, and prominent among these are the forebrain, the limbic system and the brainstem. For example, the parvocellular part of the PVH receives moderately dense projections arising from all areas of the hypothalamus (except the SON, the medial and lateral mamillary nuclei, and the magnocellular division of the PVH), from the subfornical organ and the bed nucleus of the stria terminalis, but the magnocellular divisions receive relatively few inputs from these structures (363). The PVH is also densely innervated by aminergic and peptidergic axon terminals that arise from cell bodies located in brainstem nuclei. The aminergic terminals contain NE, EPI, DA, and serotonin and, of these, the noradrenergic and adrenergic projections have been subjected to the most detailed analysis.

Aminergic innervation

The noradrenergic input to the PVH arises almost exclusively from three interrelated cell groups in

the brainstem, namely the A2 region in the nucleus of the tractus solitarius (NTS), the A1 region in the ventrolateral medulla, and the A6 area in the locus ceruleus (364, Figure 21). The fibers from the A1 region are almost entirely directed toward the magnocellular divisions and preferentially terminate on vasopressinergic cell bodies. The projections arising from the A6 area are almost entirely distributed to the parvocellular PVH, and their most prominent input is localized in the periventricular zone, an area known to contain DA-, somatostatin- and TRH-stained neurons.

The ascending adrenergic projections to the PVH are also derived from three discrete brainstem cell groups (365), namely the C1 group (in the rostral ventrolateral medulla), the C2 group (in the rostral part of the NTS), and the C3 group (in the medial longitudinal fasciculus and nucleus prepositus hypoglossi). However, in contrast to the highly differentia-

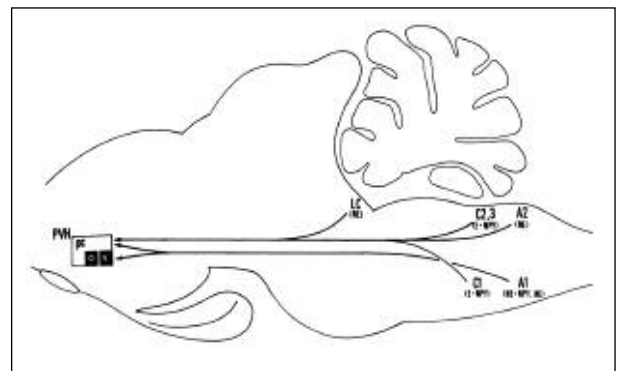


Figure 21. The brainstem catecholaminergic and NPY-immunoreactive innervation of the PVH. Schematic drawing of a sagittal section through the rat brain to indicate the dominant biochemical makeup and distribution of catecholaminergic and NPY-immunoreactive inputs from the brainstem to the PVH. Adrenergic (E) projections arise from the C1, C2, and C3 regions, are distributed overwhelmingly to the parvocellular (pc) division of the nucleus, and generally stain positive for NPY immunoreactivity. Noradrenergic (NE) projections from the locus ceruleus and A2 cell groups are also distributed primarily to the parvocellular division, but are, for the most part, NPY negative. A heterogeneous input arises from the A1 region and is distributed to both the parvocellular division and preferentially to those parts of the magnocellular division in which vasopressinergic neurons (V) predominate over oxytocinergic ones (o). One component appears also to contain NPY immunoreactivity, whereas a second one does not (reproduced with permission from ref. 282)

ted noradrenergic projections of the PVH, the projections from each of the adrenergic cell groups are very similarly distributed within the PVH, and in each case, the most dense innervation is seen in the dorsal medial parvocellular part, an area that is rich in CRF-stained neurons.

Peptidergic innervation

The PVH is also innervated by peptidergic axon terminals such as those that stain for neuropeptide Y (NPY) and galanin (GAL).

NPY is a 36-residue peptide that was originally isolated from porcine brain and has a high degree of sequence homology with peptide YY and pancreatic polypeptide (366). NPY-stained perikarya and axon terminals are widely distributed within the brain and the PVH and ARC, respectively, contain the highest density of NPY-stained axon terminals and perikarya in the brain (367-372). NPY is extensively colocalized within brainstem adrenergic neurons that project to the PVH, while its expression in noradrenergic neurons appears limited to a subpopulation of cells in the A1 group. NPY-stained projections are most dense in the anterior and medial parvocellular parts of the PVH, and these areas are known to contain CRF- and TRH-stained neurons.

GAL is a 29-residue peptide that was first isolated from porcine intestine but is also widely distributed in the central nervous system (373-379). Within the hypothalamus, GAL-stained perikarya are found in the ARC where they coexist in a large proportion of tyrosine hydroxylase (TH)-positive cells, and in the magnocellular and parvocellular PVH, where a large proportion of cells stain for both GAL and AVP. GAL-ir perikarya are also found in the locus ceruleus (A6 area) and in the caudal part of the A2 area, where they coexist within a large number of the noradrenergic neurons. In addition, the rostral parts of the A2 and C1 areas also contain GAL-positive perikarya which do not stain for TH. GAL-ir perikarya are also found in a number of other brain areas which likely do not participate in the regulation of hypothalamo-hypophysial secretion. The PVH receives a prominent galaninergic input of fibers and rostrally the most prominent inputs are confined to the anterior and peri-

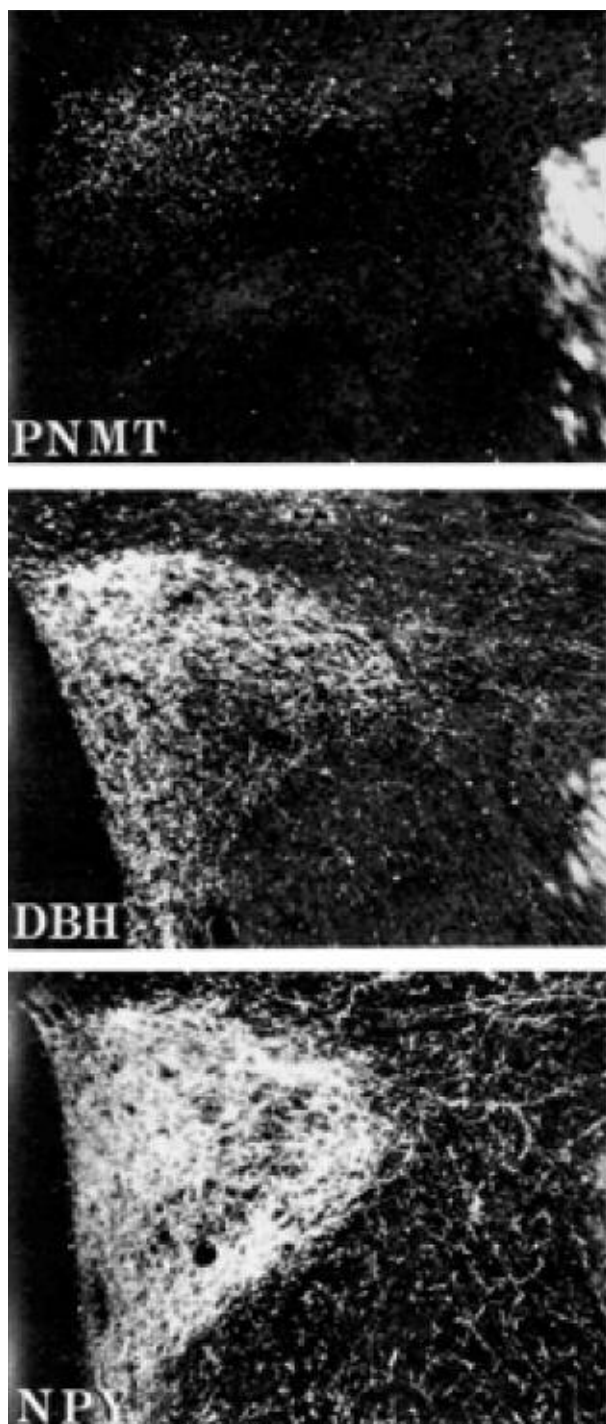
ventricular parts of the nucleus, whereas caudally the dorsal and ventral medial subdivisions are the most heavily innervated. These GAL-ir fibers arise from the A1 and A6 areas, the ARC, the dorsomedial nucleus (DMH), the LHA and the medial preoptic area (377).

Neuropeptides synthesized in the PVH

The PVH also contains a number of neuropeptides but for the purposes of this manuscript, we will limit our discussion to CRF, AVP, TRH, and VIP.

CRF

The rat brain contains about 2,000 CRF-stained perikarya distributed throughout all eight parts of the PVH, and most of these cells are found in the parvocellular division (380, 381). The medial, periventricular, and medial lateral parts of the parvocellular division contain about half of the total number of CRF-stained neurons, and these areas are known to send massive projections to the external zone of the median eminence. Furthermore the CRF neurons in the parvocellular division of the PVH may be subdivided into two populations that are distinguished by the colocalization of the AVP precursor (pro-AVP)-derived peptides AVP, the vasopressin-neurophysin (NP) or the pro-AVP C-terminal glycopeptide (382, 383). The CRF neurons receive axonal inputs that stain for NPY, TH, dopamine- β -hydroxylase, Cocaine and Amphetamine-Regulated Transcript (CART), glutamate (GLU), Glucagon like peptide-1 and Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP), indicating that they are subject to a wide variety of influences (370, 384-388, Figure 22). About 15% of the total CRF-stained population is found in those areas of the magnocellular division that predominantly contain oxytocinergic cells and in addition to the PVH, CRF-stained cells are also found in the basal hypothalamus, telencephalon, and brainstem, and these areas are involved in the functioning of the autonomic nervous system. Finally, CRF-stained cells are found scattered throughout the cerebral cortex where they are concentrated in layers II and III.



● **Figure 22.** Innervation of the PVH by PNMT, DBH and NPY immunoreactive fibers. Darkfield photomicrographs of avidin-biotin immunoperoxidase preparations to show the distribution of fibers and varicosities stained for phenylethanolamine-*N*-methyltransferase (PNMT), dopamine- β -hydroxylase (DBH), and neuropeptide Y (NPY) immunoreactivity at a similar level through the paraventricular nucleus (PVH; the third ventricle is at the extreme left of each micrograph). At this midcaudal level, basic similarities and differences in the density of each input may be appreciated, although in these thicker (30-35 μ M) sections, details of the distributions cannot necessarily be inferred. Note that the distribution of PNMT-stained elements is largely limited to a discrete part of the parvocellular division of the nucleus; few are seen in the magnocellular division. The DBH-stained projection encompasses and exceeds that localized with anti-PNMT, providing a prominent input to the magnocellular division, which is located at the right-hand margin of the nucleus at this level. The NPY-stained input encompasses and exceeds the distribution and density provided by DBH-immunoreactive inputs, providing perhaps the most prominent chemically specified input to the PVH yet described (reproduced with permission from ref. 282)

396). At least four different VIP systems exist within the brain – 1) an intracerebral cortical system; 2) one innervating the amygdala and bed nucleus of the stria terminalis; 3) a pathway originating in the SCN, and 4) another originating the midbrain central grey. VIP-stained perikarya can be visualized in the untreated animal in the limbic and neocortex, in the SCN and in the central grey of the midbrain. However, adrenalectomy and lactation combined with colchicine treatment results in the appearance of a large population of VIP-stained perikarya in the parvocellular part of the PVH and increases the number of VIP-stained fibers in the external zone of the median eminence (391). In addition, the induction of hypothyroidism also allows the detection of VIP mRNA in these PVH neurons (395, Figure 23). These findings provide an anatomical basis for the previously described role of VIP as a prolactin-releasing factor during lactation and indicate that VIP synthesis in the PVH is regulated by the thyroid status of the animal.

VIP

VIP was originally isolated from hog small intestine and subsequently found to be widely distributed in the central and peripheral nervous systems (389-

TRH

Within the hypothalamus, the TRH-producing neurons are found in the anterior, lateral, dorsal and medial parts of the parvocellular PVH, and in the an-

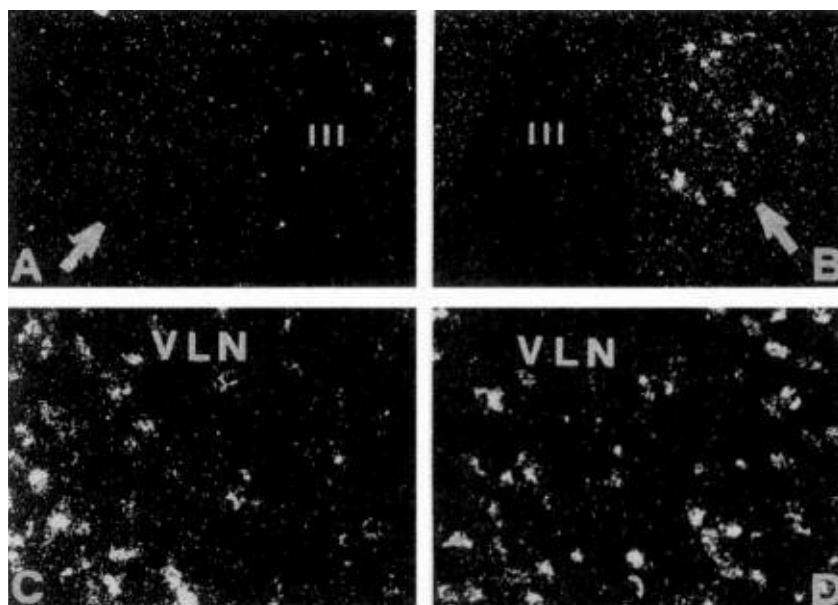


Figure 23. Effect of hypothyroidism on VIP mRNA. Darkfield photomicrograph of coronal sections through the hypothalamic paraventricular nucleus (PVN) (A,B) and thalamic ventrolateral (VLN) (C,D) nuclei on the same tissue sections. Sections were hybridized with a ^{35}S -labeled rat VIP mRNA antisense probe. Note presence of hybridized cells in the PVN only in the hypothyroid animal (B) but conspicuous absence in the euthyroid control (A). Arrow denotes the PVN; III = third ventricle (reproduced with permission from ref. 395)

terior periventricular area. Moreover, these TRH neurons receive a variety of axonal inputs including those that stain for NPY, GAL, CART, Agouti-related protein (Agrp), and α -melanocyte-stimulating hormone. These inputs originate from the ARC, the DMH, and the brainstem, indicating that TRH neurons are regulated in a highly complex manner (397-405). In addition, TRH-stained neurons are also found in the basal part of the anterior and lateral hypothalamus, the perifornical area and DMH, where they are likely to subservise functions other than the regulation of TSH and PRL secretion.

ARC

The ARC surrounds the ventral part of the third ventricle and communicates primarily with the pituitary gland, hypothalamus, limbic system, midbrain periaqueductal gray and brainstem autonomic nuclei (406, 407). In addition, the ARC contains receptors for glucocorticoids, estradiol, insulin, leptin and GH (408-413) and in this way, it integrates emotional and sensory stimuli and peripheral signals relating to an individual's metabolic status, which it may then relay to the those brain areas concerned with the regulation of the endocrine and autonomic nervous systems.

Neuropeptides synthesized in the ARC

At least fifteen neurotransmitters and neuropeptides have been found in arcuate perikarya but for the purposes of this review, we will focus on GRF and the Central Melanocortin System which includes those neurons which express NPY and Agrp, and POMC.

GRF

As noted above, GRF perikarya are located in the ARC and the GRF staining in the ARC and external zone of the median eminence is obliterated by treatment of neonatal rats with the ARC toxin monosodium GLU, indicating that the ARC is the source of GRF that enters the hypophysial-portal circulation (265, 414-417). Neurotensin is colocalized within a subset of these GRF-ir neurons, but colocalization of GRF with α -melanocyte stimulating hormone or ACTH (1-24) has not been demonstrated.

The Central Melanocortin System

The mammalian central melanocortin system is defined as a collection of circuits that include (i) ARC neurons that express NPY and Agrp (NPY/Agrp) and POMC, (ii) POMC neurons originating in the NTS

in the brainstem and (iii) downstream targets of these neurons that express the melanocortin-3 and melanocortin-4 receptors (MC3-R and MC4-R) (418, 419).

NPY/Agrp neurons

As stated above, the ARC contains the highest density of immunoreactive NPY perikarya of any area in the brain (367, 368). The ARC NPY neurons provide the major NPY-ir input to CRF-, AVP-, and TRH-stained neurons in the PVH (370, 401, 420), they project to the SON and form synapses with AVP-ir neurons (421), and they also provide ~50% of the NPY innervation to the gonadotropin-releasing hormone neurons in the medial preoptic area (422). These fiber projections provide the anatomic basis for the established roles of NPY in regulating the hypothalamic-pituitary-adrenal, -thyroid, and -gonadal axes. In addition, NPY is the most powerful orexigenic peptide known and its administration to experimental animals causes a robust and sustained increase in food intake, an effect that is partly mediated by the Y2 NPY receptor (423, 424). NPY also decreases the sympathetic outflow to brown adipose tissue in the rat and may thereby decrease the metabolic rate (425). The ARC NPY neurons are major targets for the action of insulin and leptin and these aspects are discussed below.

Agrp was isolated in 1997 and is a 132-residue peptide that is a homolog of the skin agouti peptide (426, 427). The skin agouti peptide is an antagonist of the MC1-R on melanocytes (428) whereas *Agrp* is an antagonist of brain MC3-R and MC4-R receptors (427, 429). *Agrp*-ir perikarya are found exclusively in the ARC where the peptide is colocalized within

~95% of the NPY neurons (430, 431). The ARC sends dense fiber projections to the hypothalamus and septal region and within the hypothalamus, the most dense fiber staining is seen to proceed along the third ventricle as well as in the Pv nucleus, the parvocellular PVH, the DMH, and the rostral end of the posterior nucleus (432, Figure 24). Those hypothalamic areas devoid of *Agrp*-positive fibers include the magnocellular PVH, the SON, the SCN, the ventromedial nucleus and the compact zone of the DMH (Figure 25).

POMC neurons

The POMC gene codes for the 241-residue protein POMC and is expressed in the pituitary and in many non-pituitary tissues (433-439). POMC mRNA is most abundant in the pituitary, but its content in most of the non-pituitary tissues is extremely low and the generated mRNAs are truncated, non-functional transcripts that cannot be efficiently translated. POMC is a prototypical polypeptide precursor which contains eight pairs, and one quadruplet, of basic amino acids which are potential cleavage sites for processing enzymes (Figure 26), and the nature of the POMC products in any given tissue therefore reflects which cleavage sites are used. For example, only four of the cleavage sites are used in the anterior pituitary corticotrope and the peptides produced include N-terminal peptide (NT), joining peptide (JP), ACTH, β -lipotropin (β -LPH) and a small amount of γ -LPH and β -endorphin. The ARC also expresses the POMC peptide but, in this nucleus, all the cleavage sites are used and smaller peptides are produced – NT gives rise to the γ -melanocyte-stimulating hormones (γ MSHs), ACTH yields α -MSH and CLIP (corticotropin-like intermediate lobe pepti-

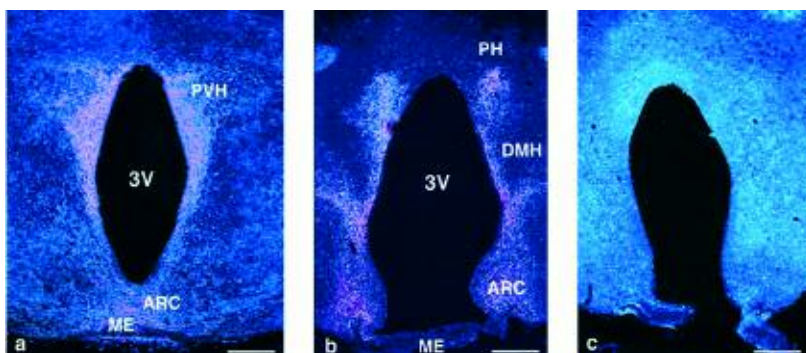


Figure 24. Immunohistochemistry demonstrates dense hypothalamic neuronal fibers expressing AGRP in the diestrous rat. AGRP immunoreactivity is found in hypothalamic fibers projecting from the ARC as well as in the PVH (a) and DMH and PH nuclei (b). Preadsorption with the immunizing peptide AGRP-(83-132) blocks the staining reaction (c). Bars, 100 μ m (reproduced with permission from ref. 432)

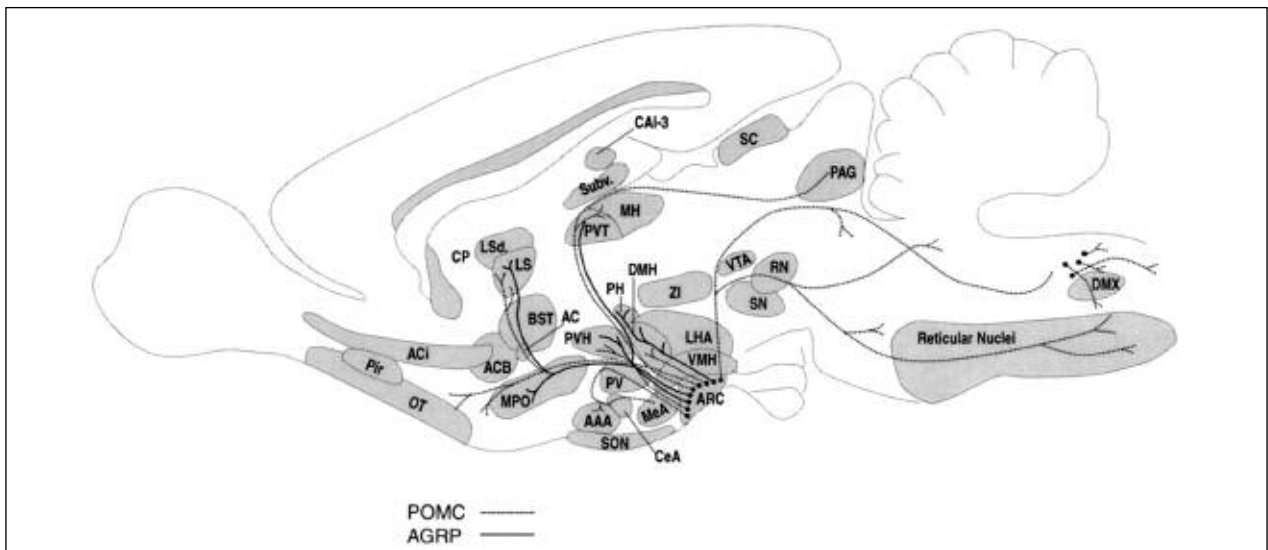


Figure 25. Schematic diagram of a sagittal view of the rat brain, illustrating the comparative distributions of POMC and AGRP neurons. AAA, anterior amygdaloid area; AC, anterior commissure; ACB, nucleus accumbens; ACi, anterior commissure, intrabulbar; ARH, arcuate nucleus of the hypothalamus; BST, bed nucleus of the stria terminalis; CA1-3, field CA1-CA3 of the hippocampus; CeA, central nucleus of the amygdala; CP, caudate putamen; DMX, dorsal motor nucleus of the vagus; LHA, lateral hypothalamic area; LSd, lateral septal area, dorsal aspect; MeA, medial amygdala; MH, medial habenula; MPO, medial preoptic area; OT, olfactory tubercle; PAG, periaqueductal gray; PH, posterior hypothalamus; Pir, piriform cortex; PV, periventricular zone; PVH, paraventricular nucleus of the hypothalamus; PVT, paraventricular nucleus of the thalamus; RN, red nucleus; SC, superior colliculus; SN, substantia nigra; SON, supraoptic nucleus; Subv, subiculum, ventral; VMH, ventromedial nucleus of the hypothalamus; VTA, ventral tegmental area; ZI, zona incerta. The locations of AGRP-immunoreactive fibers and cell bodies are based on data from the rat; fiber termini remain hypothetical. AGRP fiber distribution in the caudal brainstem was not examined in this study (reproduced with permission from ref. 432)

de), and β -LPH is processed to β -MSH, β -end₍₁₋₃₁₎, and β -end₍₁₋₂₇₎. α -MSH is an agonist of both the MC3-R and MC4-R (440,441) and is regulated by both insulin and leptin.

The CART was originally identified as an mRNA that was upregulated in rat striatum and cerebellum by the administration of cocaine and amphetamine (442). In the rat, the CART gene encodes a peptide of either 116 or 129 residues which includes a leader sequence of 27 residues, thus resulting in a mature CART peptide of either 102 or 89 residues. In contrast to the rat which contains both the long and short CART peptides, only the short form exists in humans. CART mRNA and peptides are found in many hypothalamic nuclei including the ARC where it is coexpressed in most of the ARC POMC-positive cells and regulated by leptin.

CART is a potent inhibitor of feeding and can completely override the feeding response induced by neuropeptide Y (443). CART-positive perikarya are

also found in the PVH, the SON, the lateral hypothalamic area (LHA), the DMH, the Pv nucleus, and the ventral premammillary nucleus (PMV), and CART-positive fibers are distributed throughout the hypothalamus. CART-positive fibers form synaptic contacts with TRH and CRF-stained neurons in the PVH and these findings provide the anatomical basis for the involvement of the peptide in the regulation of both the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenal axes (385, 444-449).

Hormonal regulation of NPY/Agrp and POMC/CART neurons by insulin and leptin

Insulin

Several lines of evidence indicate that insulin is a key peripheral hormonal signal that regulates food intake and body fat mass (450-452). First, the plasma $t_{1/2}$ of insulin is 2-3 minutes, thus rendering the hor-

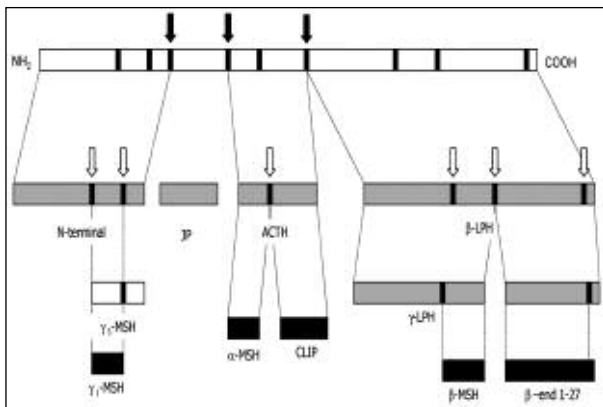


Figure 26. POMC posttranslation processing by PC1 (black arrow) and PC2 (clear arrow) at dibasic cleavage sites (solid line). Tissuespecific expression results in a different range of peptides produced in the anterior pituitary (■) compared with the hypothalamus (■). (reproduced with permission from Coll AP, Farooqi IS, Challis BG, Yeo GS, O'Rahilly S: *J Clin Endocrinol Metab* 2004; 89:2557-2562)

mone capable of responding rapidly to changes in metabolism and providing the brain with minute-to-minute information of an individual's physiological state. Second, insulin receptors are widely distributed in the cen-

tral nervous system and are found in particularly high concentrations in those brain regions involved in the regulation of food intake and body weight (453, 454). Third, insulin gains entry into the brain via a saturable transport process that moves the hormone from the plasma into brain interstitial fluid (455). Fourth, the intracerebroventricular (icv) administration of insulin reduces food intake and this effect may be due to its ability to decrease NPY/Agrp and increase POMC gene expression in the ARC (456-458). The effects of insulin in the ARC are mediated by tyrosine phosphorylation of the insulin receptor, IRS-1 and -2, increased binding of activated IRS-1 and -2 to the regulatory subunit of PI3-kinase and activation of protein kinase B/Akt (459). It is noteworthy that the insulin-induced increase in PI3-kinase activity preferentially occurs in IRS-2-containing neurons and that its inhibitory effect on food intake is blocked by PI3-kinase inhibitors (Figure 27).

Leptin

Leptin, a 167-residue peptide that is the product of the *ob* gene, is derived almost exclusively from adi-

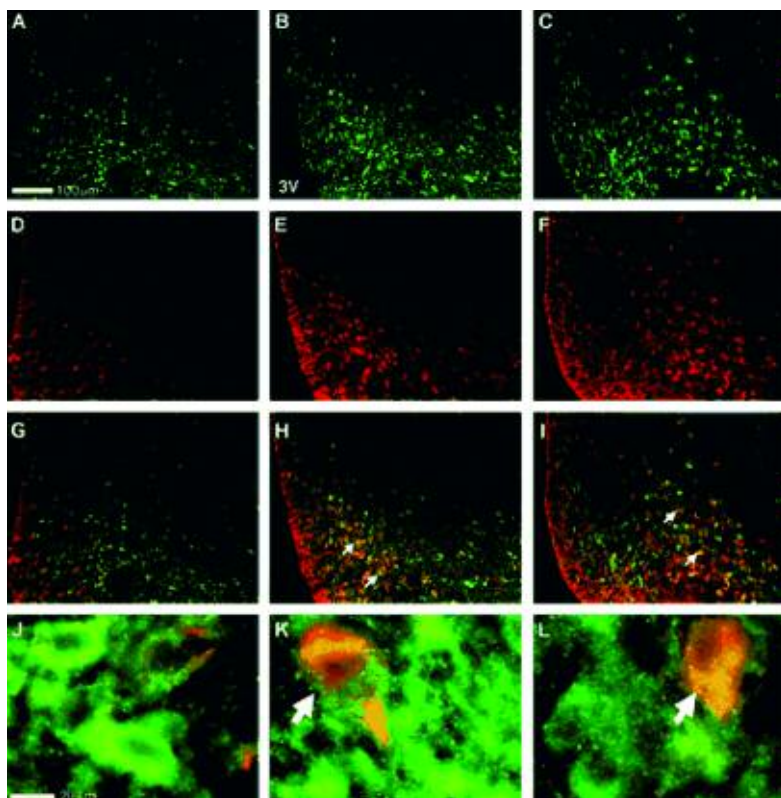


Figure 27. Insulin enhances PIP₃ immunoreactivity primarily in IRS-2-expressing arcuate nucleus neurons (A-I: x20 magnification, third ventricle at lower left of frame; J-L: x100 view of ARC neurons). Rats were treated with intracerebroventricular vehicle (A, D, G, and J), intracerebroventricular insulin (10mU, 5 min; B, E, H, and K), or peripheral insulin (5 units i.p., 15 min; C, F, I, and L); the sections of arcuate nucleus immunostained for IRS-2 (A-C) and PIP₃ (D-F); and the images merged at low (G-I) and high power (J-L). IRS-2 immunoreactivity was detected specifically in neurons of the arcuate nucleus with all treatments (A-C), and with no staining detected elsewhere. Insulin treatment, either intracerebroventricularly (E) or peripherally (F), induces increased PIP₃ immunoreactivity compared with vehicle treatment (D). Enhanced PIP₃ immunoreactivity occurs primarily in IRS-2-positive neurons (G-I). Especially at high magnification, PIP₃/IRS-2 double-positive neurons are identified in insulin-treated arcuate nucleus neurons (K and L), whereas few if any are observed in vehicle-treated rats (J). 3V, third cerebral ventricle (reproduced with permission from ref. 459)

pose tissue and is secreted in a pulsatile manner into the systemic circulation (460, 461). Leptin is an important peripheral signal that regulates energy balance since both peripheral and icv administration of the hormone reduces food intake and body weight (462, 463).

The leptin receptor (ObR) belongs to the cytokine receptor class I super-family (464) and five alternatively spliced forms with different carboxy-terminal lengths (a-e) have been identified (465). The expression of the short leptin receptor isoform, ObRa, is highest in the choroid plexus and microvessels where it may be involved in receptor-mediated transport of the hormone across the blood-brain barrier and in the clearance of leptin from the cerebrospinal fluid (466-469). The long form of the leptin receptor, ObRb, is expressed in varying concentrations in several brain nuclei (470, 471). Within the hypothalamus, dense mRNA expression is found in the ARC, DMH, ventromedial hypothalamus (VMH), and ventral premamillary (PMV) nuclei, moderate expression is found in the Pv and LHA, and lower levels still are found in the PVH. Many of these areas are involved in the regulation of feeding behavior and animals bearing a selective neuron-specific deletion of the ObR develop obesity, indicating that most, if not all, of leptin's weight-reducing effects are due to its actions in the brain (472).

Activation of ObRb results in activation of the associated Jak2 tyrosine kinase and subsequent tyrosine phosphorylation of ObRb (473, 474). Two important tyrosine residues that are phosphorylated during receptor activation are Tyr⁹⁸⁵ and Tyr¹¹³⁸ which mediate distinct signaling pathways. Tyr⁹⁸⁵ binds to the src homology 2 (SH2)-domain protein, SH2-domain phosphotyrosine phosphatase (SHP-2), and leads to activation of the extracellular signal-regulated kinase (ERK) and induction of *c-fos* expression. Tyr¹¹³⁸ binds to STAT3 proteins which become tyrosine phosphorylated by Jak2, dissociate in the cytoplasm to form dimers, and finally translocate to the nucleus where they regulate gene transcription (Figure 28, 475). One of the genes induced by STAT3 is that which codes for SOCS3 which functions as a major feedback inhibitor of ObRb signaling (476). In addition, the effects of ObRb activation on food intake, body weight and the Jak-STAT pathway re-

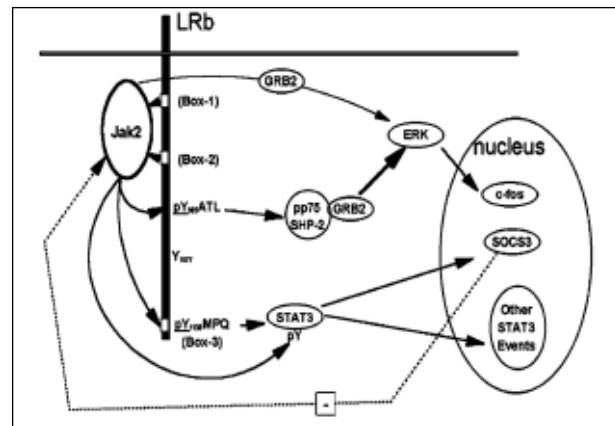


Figure 28. Model of LRB signaling. Murine LRB contains three intracellular tyrosine residues (shown with surrounding amino acids) in addition to the conserved Box 1 and Box 2 motifs required for interaction with Jak2. Upon ligand stimulation, the associated Jak2 tyrosine kinase becomes activated, autophosphorylating and phosphorylating Tyr⁹⁸⁵ and Tyr¹¹³⁸ of the LRB. Phosphorylated Tyr¹¹³⁸ recruits STAT3, which is then tyrosine-phosphorylated by Jak2, whereupon it translocates to the nucleus to mediate the transcription of *socs3* and other genes. SOCS3 ultimately feeds back upon and inhibits Jak2/LRB signaling (*dotted line*). Phosphorylated Tyr⁹⁸⁵ recruits SHP-2, which is then tyrosine-phosphorylated by Jak2. Phosphorylated SHP-2 (identical to pp75) binds GRB-2 and mediates the majority of ERK activation during LRB signaling. An additional minor amount of GRB-2 binding and ERK activation is mediated directly by Jak2 (*thin line*). The activation of ERK results in the transcription and accumulation of *c-fos* message (reproduced with permission from ref. 475)

quire an intact PI3-kinase-phosphodiesterase 3B-cyclic AMP signaling pathway, since these effects are blocked by a phosphodiesterase 3 inhibitor (477, 478).

The weight-reducing effects of leptin are mediated by stimulation of the anorexigenic POMC neurons and inhibition of the orexigenic NPY/AgRP neurons in the ARC (479-485). Leptin increases the frequency of action potentials in POMC neurons by causing depolarization through a nonspecific cation channel and by reducing the inhibition exerted by local NPY/ γ -aminobutyric acid neurons (484). As judged by its ability to activate PI3K, leptin increases the membrane accumulation of PI3K in POMC neurons but decreases PI3K accumulation in AgRP neurons (Figure 29, 485). Moreover, this latter effect of leptin on AgRP neurons is indirect, since it is blocked by

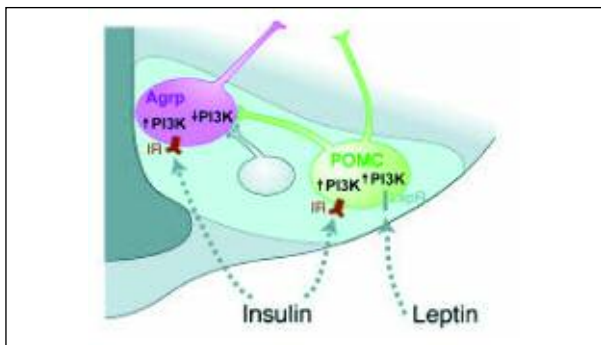


Figure 29. Unifying mechanism for leptin modulation of key arcuate nucleus neurons in which PI3K activity is a mediator and/or marker of neuronal activation and neuropeptide release in both AgRP (pink) and POMC (green) neurons. The effects of insulin on PI3K activity are direct in both neuronal subtypes, but the effects of leptin on PI3K activity in AgRP neurons require synaptic transmission from POMC or other (gray) inhibitory synaptic neurons. IR, insulin receptor; LepR, leptin receptor (reproduced with permission from ref. 485)

inhibitors of synaptic transmission. Although insulin, like leptin, increases PI3K accumulation in POMC neurons, it also increases PI3K accumulation in AgRP neurons and neither of these actions are affected by inhibitors of synaptic transmission.

These results provide a new model to explain how leptin may exert different effects on these two cell types and suggest that the parallel effects of leptin and insulin on energy balance could be integrated at the level of the POMC neuron.

DMH

Connections

The DMH has been associated in some way with almost every goal-directed behavior and visceral response associated with the hypothalamus. The DMH lies adjacent to the third ventricle caudal to the PVH, dorsal to the VMH, and ventral to the zona incerta. The majority of the inputs to the DMH arise in the hypothalamus, although there are a few significant inputs from the telencephalon and brainstem (Figure 30, 486). With the exceptions of the magnocellular preoptic nucleus, the magnocellular parts of the PVH, the SON, and the medial and lateral mamillary nuclei, each major hypothalamic nucleus and area provides inputs to the DMH. The major projections from the DMH are also intrahypothalamic and follow three distinct ascending pathways -a) *paraventricular*, b) *ventral*, and c) *lateral*. Within the hypothalamus, the most densely innervated areas are the dorsal and ventral medial parvocellular parts of the PVH, other dorsal regions of the periventricular zone, the preoptic SCN, and the parastriatal nucleus (487).

Neuropeptides synthesized in the DMH

The DMH also contains neuropeptide perikarya and fibers but for the purposes of this discussion, we will only focus on CRF and NPY since their genes have been shown to be regulated by exercise.

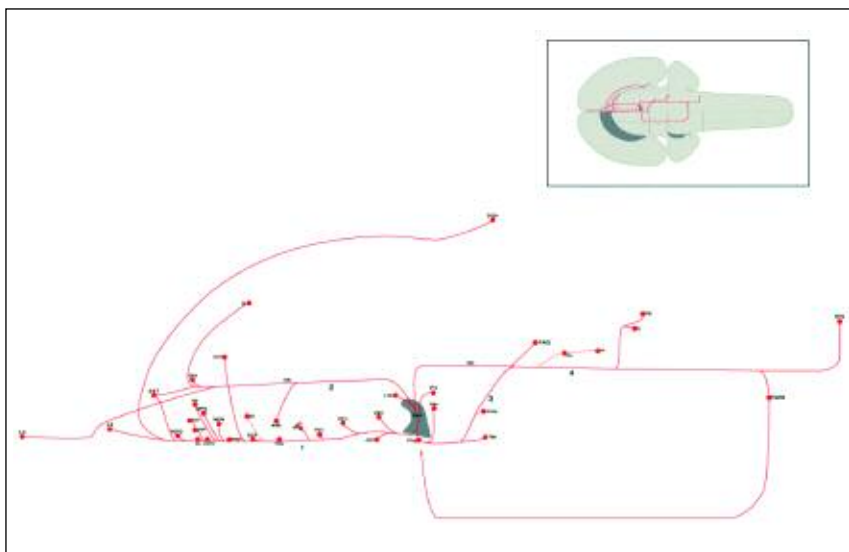


Figure 30. General organization of projections to the DMH. Inputs primarily utilize three descending pathways: *periventricular* and *medial* (1) and *lateral* (2), and two major ascending pathways: *midbrain periventricular* (3) and *brainstem lateral* (4). Pathways that were observed in our control injections, or reliably reported in the literature, are represented by a solid line. Regions for which the pathway is uncertain are represented by a dashed line (reproduced with permission from ref. 486)

CRF

Several studies have shown that food intake and body weight are significantly decreased when rats are allowed free access to a running wheel (488,489). In addition, 42 h of running wheel access augments CRF gene expression in cell bodies located in the dorsal aspect of the DMH, but has no effect on CRF gene expression in the PVH (Figure 31, 490). Since the icv administration of a CRF antagonist specifically prevents the effects of exercise on meal size (491), the findings suggest that exercise induces endogenous CRF release leading to a reduction in meal size and food intake. Although the PVH and central nucleus of the amygdala (CeA) are important sites that mediate some of the central actions of CRF, lesions of these nuclei have no effect on exercise-induced anorexia, indicating that these brain areas do not play a role in mediating this response (492, 493). To date, the exact mechanisms by which exercise initially activates CRF gene expression in the DMH remain unknown.

NPY

Physical exercise also increases NPY mRNA expression in the DMH and ARC (400, 494), but in contrast to the time course with which running wheel

access increases DMH CRF gene expression, DMH NPY gene expression only increases after 7-days of exercise. Although DMH CRF gene expression is still elevated after 7-days of exercise, the CRF-mediated, exercise-induced anorexia subsides and the food intake in exercised animals closely approximates that seen in sedentary animals. It therefore appears that the increase in NPY gene expression seems to override the effect of CRF on food intake at this time.

Postulated mediation by Musculin of exercise-induced GH, ACTH and PRL secretion

A number of studies in man have demonstrated that physical exercise has no discernible acute or chronic effects on serum leptin concentrations (495-499), suggesting that it is unlikely that leptin plays a significant role in mediating the effects of exercise on anterior pituitary secretion. Furthermore, although insulin may stimulate anterior pituitary hormone secretion indirectly by virtue of the hypoglycemia that results from excessive insulin production or administration, the occurrence of hypoglycemia implies an underlying pathophysiological state, and is not a usual concomitant of physical exercise. Moreover plasma insulin levels decline, rather than increase, during exercise, and taken together these findings also tend to exclude a significant role for

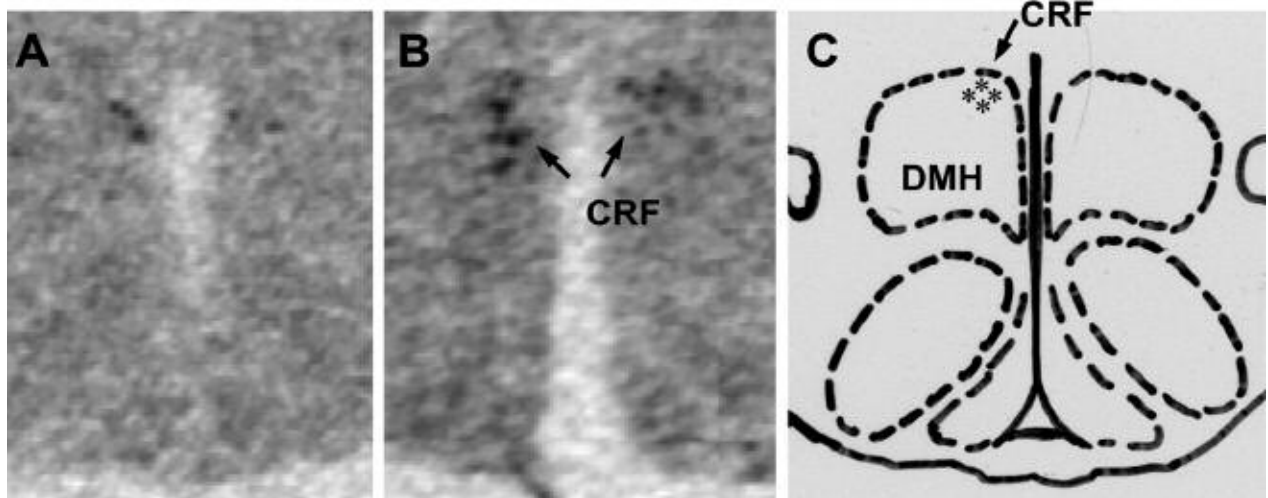


Figure 31. Effect of exercise on CRF gene expression in the DMH. In situ hybridization of CRF with ^{35}S -labeled-CRF antisense riboprobe. CRF gene expression was detected very lightly in sedentary rats (A), CRF was highly expressed in the DMH in voluntary exercising rats (B), and the induction of DMH CRF expression was mainly localized to the dorsal region of the DMH (B and C) (reproduced with permission from ref. 490)

insulin as a mediator of exercise-induced pituitary hormone secretion. Considerations such as these set the stage for the possible involvement of additional factor(s) as mediators of exercise-induced pituitary hormone secretion. We therefore postulate that:

- *Musculin concentrations in the systemic circulation increase during physical exercise in increments that correlate directly with the exercise intensity.*
- *Musculin acts directly on the brain to mediate the anterior pituitary hormone and appetite responses to exercise.*

GH

From the aforementioned review, we postulate that:

- *Musculin acts directly on ARC GRF neurons to increase the synthesis and release of GRF into the hypophysial-portal circulation.*

Since physical exercise also increases Prepro-GAL gene expression in the Locus Ceruleus (A6 area, 500), we postulate that:

- *Musculin binds directly to GAL neurons in the A6 area and stimulates GAL synthesis and secretion.*

Since the A6 area does not lie outside the blood-brain barrier (501), we postulate that, like insulin and leptin (455, 466-469, 502-505),

- *Musculin may gain access to the brain by a receptor-mediated active transport mechanism.*

Studies in the rat and in man suggest that GAL stimulates GH secretion by both increasing GRF release and by inhibiting SRIF secretion (506-513). Since axons derived from A6 GAL-immunoreactive neurons project to the periventricular part of the PVH (377) and synapse directly with those SRIF neurons that project to the median eminence (514), we propose that:

- *Musculin also increases GH secretion by enhancing the inhibitory galaninergic regulation of hypothalamic SRIF release.*

These postulates are schematically illustrated in Figure 32.

ACTH

As previously stated, it is currently accepted that the hypothalamus only provides a unidirectional, sti-

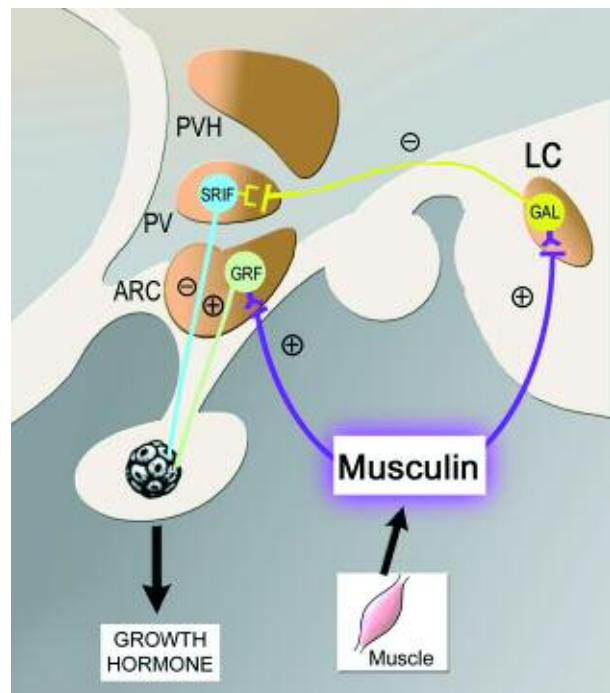


Figure 32. A hypothetical scheme depicting the mechanisms by which *Musculin* may mediate the Growth Hormone response to exercise. It is suggested that *Musculin* may bind directly to ARC GRF neurons and stimulate GRF synthesis and its release into the hypophysial-portal circulation. In addition, it is hypothesized that *Musculin* binds to galanin neurons in the locus ceruleus and increases galanin gene expression at this site. These galanin neurons give rise to axons which innervate SRIF neurons in the periventricular area. The galaninergic regulation of SRIF is inhibitory and the resultant reduction in SRIF secretion into hypophysial-portal blood would also be expected to facilitate Growth Hormone secretion. ARC, arcuate nucleus; GAL, galanin; GRF, Growth Hormone-Releasing Factor; LC, locus ceruleus; PV, periventricular area; PVH, paraventricular hypothalamic nucleus; SRIF, somatostatin

mulatory regulation of ACTH secretion and that this is predominantly mediated by the neuropeptides CRF and AVP. In turn, the hypothalamic CRF and AVP neurons that project to the median eminence receive noradrenergic and adrenergic inputs derived from the brainstem and in previous studies, we have shown that the noradrenergic input stimulates the release of both CRF and AVP into the hypophysial-portal circulation of the conscious sheep (515). Moreover, physical exercise has also been shown to increase the concentrations of NE and its metabolite 3,4-dihydroxyphenylglycol (DHPG) in the pons-medulla (516, 517). As

previously described, the noradrenergic innervation of the PVH is derived from the A1, A2, and A6 areas, but since the A1 NE neurons mainly project to the magnocellular subdivisions of the PVH which are not concerned with HPA axis regulation and since NE turnover in the A6 area is not affected by exercise (500), we postulate that:

- *Musculin binds to NE neurons in the A2 area where it stimulates NE synthesis, thereby increasing the stimulatory noradrenergic regulation of those hypothalamic CRF and AVP neurons concerned with regulation of the HPA axis.*

The paraventricular hypothalamic CRF and AVP neurons concerned with HPA axis regulation also receive peptidergic inputs and a prominent contribution is made by NPY axons that are mainly derived from ARC NPY neurons (370). Since physical exercise increases ARC NPY gene expression and since NPY activates the HPA axis in a number of species (515, 518, 519) by increasing CRF and AVP secretion into the hypophysial-portal circulation (Figure 33, 515), we postulate that:

- *Musculin binds to ARC NPY neurons where it increases NPY synthesis, thereby increasing the stimulatory NPYergic input to those hypothalamic CRF and AVP neurons that regulate the HPA axis.*

These postulates are illustrated schematically in Figure 34.

PRL

As previously mentioned, prolactin secretion by the anterior pituitary is tonically inhibited by DA and stimulated by a number of hypothalamic releasing factors such as VIP, TRH, and OT. A number of studies have shown that the icv administration of GAL increases the release of VIP from periventricular structures into the cerebrospinal fluid (520, 521). Since the icv administration of GAL also increases prolactin secretion which is attenuated by the concomitant icv administration of a VIP antiserum (520-523), the findings support the hypothesis that GAL stimulates prolactin secretion by increasing the hypothalamic release of VIP. The VIP neurons that project to the median eminence are concentrated in the medial parvocellular subdivision of the PVH (345) and this area,

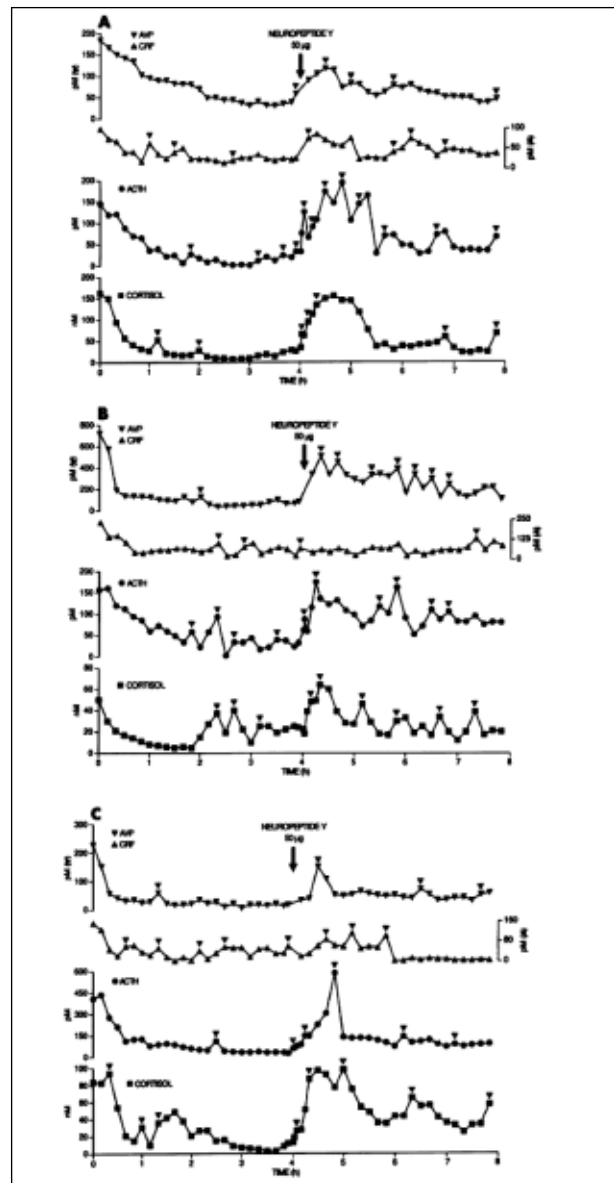


Figure 33. Activation of the hypothalamic-pituitary-adrenal axis by neuropeptide Y. The effect of neuropeptide Y (50 µg icv) on plasma CRF, AVP, ACTH, and cortisol levels in three ewes (reproduced with permission from ref. 515)

together with the periventricular and anterior parvocellular subdivisions of the PVH, receive a prominent input of galanergic fibers that are derived from the A6 area (377).

Since exercise increases GAL biosynthesis in the A6 area, we postulate that:

- *Musculin may bind to the GAL neurons in the A6 area and stimulate GAL synthesis and secretion.*

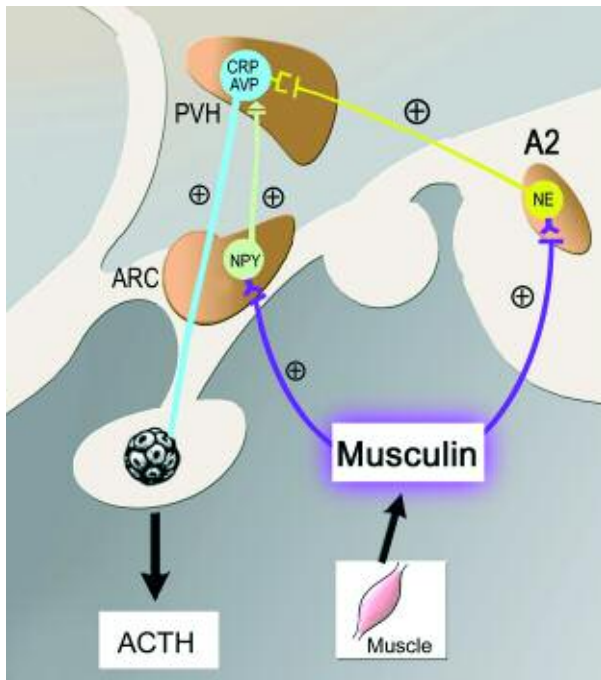


Figure 34. A hypothetical model of the mechanisms by which *Musculin* may mediate the Adrenocorticotropin response to exercise. It is suggested that *Musculin* binds to ARC neuropeptide Y neurons and increases NPY synthesis and secretion. ARC neuropeptide Y neurons project heavily to the PVH where they form synaptic contacts with CRF and AVP neurons. Neuropeptide Y stimulates CRF and AVP release into the hypophysial-portal circulation and thus increases adrenocorticotropin secretion. In addition, it is postulated that *Musculin* binds to noradrenergic (NE) neurons in the A2 area and increases NE synthesis. The A2 noradrenergic neurons give rise to axons which innervate the PVH and form synapses with CRF and AVP neurons. NE also stimulates CRF and AVP release into the portal circulation and thus increases adrenocorticotropin secretion. ACTH, adrenocorticotropin; ARC, arcuate nucleus; AVP, arginine vasopressin; A2, nucleus of the tractus solitarius; NE, norepinephrine (noradrenaline); NPY, neuropeptide Y; PVH, paraventricular hypothalamic nucleus

- *The increased galaninergic drive may stimulate the hypothalamic release of VIP from the PVH and increase the secretion of prolactin by the lactotropes of the anterior pituitary.*

As previously stated, prolactin secretion is tonically inhibited by tuberoinfundibular dopaminergic (TIDA) neuron secretion but, to our knowledge, *in vivo* microdialysis studies of the effects of physical exercise on DA secretion from ARC TIDA neurons have yet to be performed. However, it has been shown

that physical exercise acutely increases the release of DA, NE, and GLU from the rat striatum (524, 525), and although the mechanisms underlying these effects remain unclear, it is conceivable that exercise could similarly affect TIDA neuronal activity. Since activation renders TIDA neurons susceptible to the inhibitory effects of GAL (526), we postulate that:

- *Musculin may bind directly to and activate ARC TIDA neurons.*
- *Musculin-induced activation may render ARC TIDA neurons susceptible to the inhibitory effects of GAL, thereby reducing the dopaminergic drive to the lactotropes and thus increasing prolactin secretion.*

These postulates are illustrated schematically in Figure 35.

Postulated mediation by Musculin of exercise-induced anorexia

To date, the earliest described change in hypothalamic neuropeptide gene expression in response to short-term (42 h) exercise is a singular 1.5-2.0-fold increase in CRF mRNA in the dorsal DMH (490), there being no discernible effects on ARC neuropeptide gene expression. However, long-term exercise causes a 5-fold induction of DMH CRF mRNA, it subsequently induces NPY gene expression in the DMH, and increases ARC NPY and POMC mRNAs (490, 527).

Several lines of evidence support the suggestion that the early induction of DMH CRF mRNA in response to short-term exercise cannot be easily ascribed to the actions of leptin or insulin. Firstly, although the DMH does contain ObRs and responds to intravenously injected leptin with an induction of neuronal Fos immunoreactivity (ir), the Fos-ir is characteristically observed in the caudal portion of the ventral subdivision, rather than in the dorsal part of the nucleus where the CRF neurons are located (528). Secondly, most studies in man suggest that short-term physical exercise appears to have no discernible effect on serum leptin concentrations, although this statement may not be applicable to rodents. Thirdly, although long-term exercise has been shown to cause

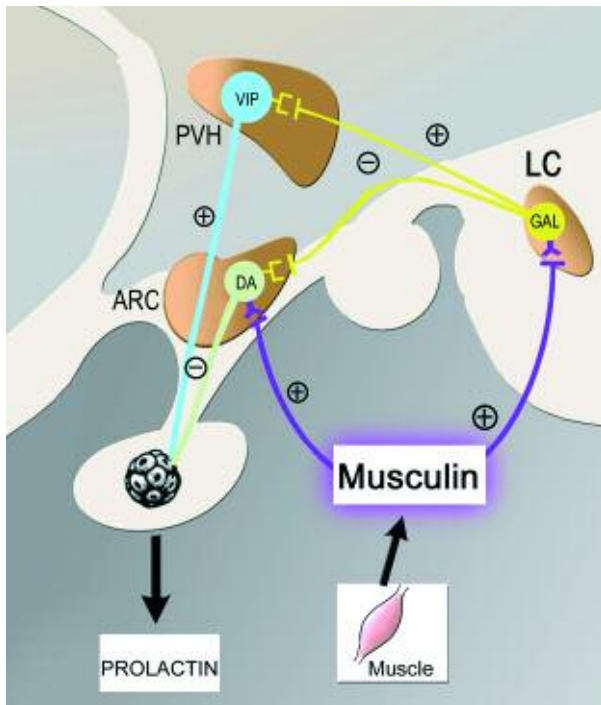


Figure 35. A hypothetical model of the mechanisms by which *Musculin* may mediate the Prolactin response to exercise. It is suggested that *Musculin* binds to galanin neurons in the locus ceruleus and increases galanin gene expression. The galanin neurons give rise to axons which innervate the paraventricular hypothalamus where they form synaptic contacts with vasoactive intestinal polypeptide neurons. The galaninergic regulation of vasoactive intestinal polypeptide is stimulatory in nature and since vasoactive intestinal polypeptide is a prolactin-releasing factor, this mechanism would be expected to increase prolactin secretion. In addition, it is suggested that *Musculin* binds to and activates ARC tuberoinfundibular dopamine (TIDA) neurons. Activation of TIDA neurons renders them susceptible to the inhibitory effects of galanin, and since dopamine tonically inhibits the release of prolactin, a posulated net reduction in dopaminergic tone would also be expected to increase prolactin secretion. ARC, arcuate nucleus; DA, dopamine; GAL, galanin; LC, locus ceruleus; PVH, paraventricular hypothalamic nucleus; VIP, vasoactive intestinal polypeptide

a ~20% reduction in serum insulin concentrations, the magnitude of this effect has not been found to be statistically significant (494). Moreover, the profound insulinopenia that characterizes the diabetes resulting from the administration of the islet β -cell toxin, streptozotocin, causes changes in ARC gene expression, but does not seem to affect neuropeptide expression in the DMH (529). From these considerations, we suggest that:

- The exercise-induced changes in DMH CRF gene expression are unlikely to be mediated by leptin or insulin.
- *Musculin* may constitute a separate non-leptin, non-insulin hormonal pathway that mediates the exercise-induced changes in DMH neuropeptide gene expression.
- *Musculin* binding and activation of DMH CRF neurons may constitute an early event in the mediation of exercise-induced anorexia.

As previously noted, long-term exercise causes a 5.0-fold induction of DMH CRF mRNA and also increases ARC POMC gene expression. A number of immunohistochemical studies have shown the presence of abundant α -MSH fibers and terminals within the DMH (530, 531). Since retrograde studies have shown that the majority of inputs to the DMH arise in the hypothalamus (486), it seems reasonable to conclude that ARC POMC neurons are the major source of α -MSH fiber projections to the DMH. In recent studies, Lechan and coworkers have sought to determine the DMH neuronal subpopulations that project to the PVH (531). When cholera toxin β -subunit was injected into the PVH, ~65% of all the DMH neurons that were retrogradely labeled were found in the medial portion of the ventral subdivision (DMHv) and ~26% were diffusely distributed in the dorsal subdivision (DMHd). Moreover, ~39% of the DMHd-labeled cells were contacted by α -MSH-stained axon terminals. Since CRF-stained neurons are located in the DMHd and project to the PVH (490, 532), it is possible that some of these retrogradely labeled cells were CRF neurons. Moreover, recent studies have demonstrated a functional link between the central melanocortin system and CRF-producing neurons, albeit in the PVH (533, 534). The MC4-R is expressed in ~10-15% of the CRF neurons in the PVH and the icv administration of α -MSH causes phosphorylation of the cAMP response element binding protein (CREB) in CRF (and TRH) neurons in several subdivisions of the PVH (533). Furthermore, the administration of the melanocortin agonist, MTII, rapidly increases CRF hnRNA, increases plasma corticosterone levels, and causes anorexia (534). The DMH is also known to express the MC4-R mRNA (535, 536) and although, to our knowledge, anatomical and functional stu-

dies of a possible melanocortin-CRF interaction have yet to be performed in the DMH, we postulate that:

- *Musculin may bind to ARC POMC neurons and increase POMC gene expression.*
- *The resultant increase in ARC POMC mRNA expression may secondarily potentiate CRF gene expression in the DMH by increasing an α -MSH-mediated stimulation of the DMH CRF gene.*

As previously stated, long-term exercise induces DMH NPY gene expression in the DMH (490, 527), and it is noteworthy that this phenomenon has also been demonstrated in the following pathophysiological and physiological states - firstly, it has been observed in several rodent models of obesity including obese MC4-R^{-/-} and obese *A^y* mice (537-539). Since these animals represent two examples of the melanocortinergic obesity syndrome and are respectively characterized by diminished or absent melanocortin signaling, it has been proposed that des-acetyl- α -MSH released at MC4-R-containing synapses in the DMH normally inhibits NPY gene expression in this nucleus (537). However, the absence of DMH NPY gene expression in nonobese *A^y* animals indicates that abrogation of melanocortinergic signaling alone is not sufficient to cause the phenomenon. Secondly, DMH NPY gene expression has been found after long-term food restriction (494), after the administration of naloxone, which also causes mild anorexia and a reduction in food intake (540), and in diet-induced obesity (541).

Thirdly, DMH NPY gene induction occurs during lactation in the rat, in which 3 h of suckling is sufficient to activate DMH NPY gene expression, but 24 h of suckling is required to increase NPY mRNA in the caudal portion of the ARC (542). The studies by Smith and colleagues (542-545) also suggest that the DMH NPY gene is inhibited by melanocortin signaling since lactation also reduces ARC POMC mRNA (545), and bilateral injections of an MC4-R/3-selective agonist (melanotan II) into the DMH of the lactating rat greatly attenuates the induction of DMH NPY gene expression and the suckling-induced hyperphagia (536). A schematic diagram summarizing this postulated sequence of events is shown in Figure 36.

As noted, long-term physical exercise *increases* both ARC NPY and POMC mRNAs (490, 527). Moreover, this pattern of ARC neuropeptide gene expression seems unique, since it differs from that caused by leptin and insulin, which both increase ARC POMC mRNA and decrease ARC NPY mRNA (451-453, 457, 458, 479, 484, 546), and suckling, which increases ARC NPY mRNA and decreases ARC POMC mRNA. Therefore, although a role for melanocortin signaling in the induction of DMH NPY mRNA seems established in obese rodent models of melanocortinergic obesity and lactation as described above, the rise in ARC POMC mRNA during long-term exercise renders it difficult to invoke withdrawal of melanocortinergic signaling in the DMH as a pri-

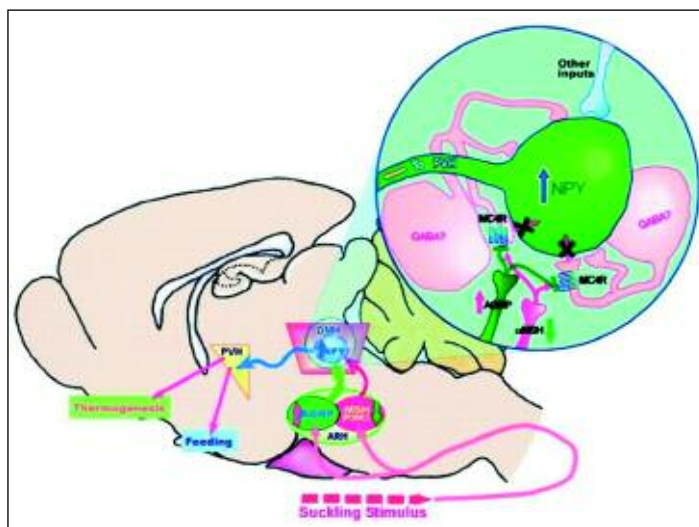


Figure 36. Schematic diagram summarizes the proposed hypothesis for MC4R-mediated activation of DMH NPY neurons and the hyperphagic response during lactation. During lactation, ARH AgRP/NPY input to the DMH is elevated whereas ARH POMC tone into the DMH is reduced. Most of these inputs likely terminate on inhibitory interneurons expressing MC4R (i.e. GABAergic) in the DMH. The inset depicts MC4R signaling. The increased AgRP input in combination with reduced POMC input from the ARH causes a reduction in MC4R signaling, leading to a decrease in GABAergic inhibition on the DMH NPY neurons, resulting in activation of NPY mRNA expression during lactation. The activated NPY neurons in the DMH may be involved in a number of modulations during lactation, including hyperphagia and energy expenditure, probably via projection to the PVH (reproduced with permission from ref. 536)

primary mechanism responsible for the induction of DMH NPY gene expression by exercise, and points towards mechanism(s) other than those mediated by the MC4-R.

It is possible that the induction of DMH NPY mRNA by long-term physical exercise is the result of metabolic or hormonal effects of the exercise and this suggestion is supported by studies which have examined the effects of acute food deprivation or chronic food restriction in the rat (547). These studies have demonstrated that both experimental methods of caloric deprivation elevate ARC NPY and decrease ARC POMC gene expression, but only chronic food restriction induces NPY gene expression in the DMH. Although acute food deprivation and chronic food restriction both cause weight loss and identical reductions in serum leptin, plasma glucose and insulin levels are only reduced by acute food deprivation. These data indicate that ARC and DMH NPY mRNAs are differentially regulated, and unlike ARC NPY mRNA which is responsive to short-term alterations in food intake, DMH NPY gene expression may only respond to long-term alterations in energy intake or expenditure. Moreover, the findings also suggest that DMH NPY gene expression is not regulated by leptin, a conclusion which is further strengthened by the lack of colocalization of the ObRs in DMH NPY neurons (547). Finally, studies in the lactating rat have shown that DMH NPY neurons may also be hormonally regulated by prolactin, since the suckling-mediated induction of DMH NPY gene expression is significantly attenuated when the associated hyperprolactinemia is prevented by the dopaminergic agonist, bromocriptine (548).

It is possible that several etiological factors could underly the exercise-induced increase in ARC NPY and POMC mRNAs. Firstly, this pattern of ARC gene expression could be entirely caused by the direct effects of *Musculin*. *Musculin* binding to ARC POMC neurons could increase PI3K, as has been demonstrated for leptin and insulin, and this would result in a model whereby the effects of leptin, insulin, and *Musculin* are integrated at the level of these anorexigenic neurons (Figure 37). If *Musculin* also activated ARC NPY/Agrp neurons by synaptic interaction with POMC neurons, as has been demonstrated for leptin

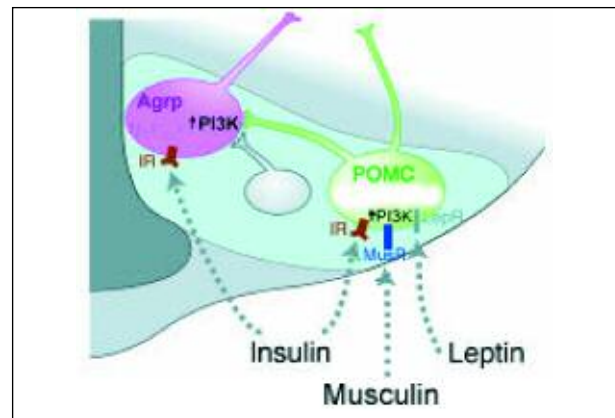


Figure 37. A schematic representation of the modulation of arcuate nucleus neurons by leptin and insulin, and postulated modulation by *Musculin*. This figure is a modification of Figure 29 and PI3K activity is depicted as a mediator and/or marker of neuronal activation and neuropeptide release in both Agrp (pink) and POMC (green) neurons. For the sake of clarity, the effects of insulin and leptin on PI3K activity have been omitted, but are identical to that shown in Figure 29. The figure proposes that the effects of *Musculin* on Agrp neurons are synaptically mediated from POMC or other neurons. However, in contrast to leptin which decreases PI3K activity in Agrp neurons, it is suggested that *Musculin* may increase PI3K in these neurons. IR, insulin receptor; LepR, leptin receptor; MusR, *Musculin* receptor

(485), then one would predict that PI3K activity would increase in response to *Musculin*, thereby opposing the actions of leptin. However, one could also envisage a model whereby *Musculin* bound both POMC and ARC neurons separately and increased PI3K activity in both neuronal subtypes (Figure 38).

Finally, it also remains possible that the exercise-induced increases in ARC NPY and POMC mRNAs could represent a combination of the direct effects of *Musculin* binding to POMC neurons and the indirect effects of the metabolic and hormonal responses to exercise on NPY/Agrp neurons. For example, as previously detailed, exercise in man and rodents is associated with a reduction in serum insulin concentrations. Moreover, although studies in man have generally suggested that acute exercise has little effect on serum leptin levels, exhaustive exercise in man and long-term exercise in rodents does reduce leptin concentrations (549-551). As noted, ARC NPY/Agrp neurons respond to leptin and insulin withdrawal with an upregulation of NPY mRNA expression and these mechanisms could theoretically account for the in-

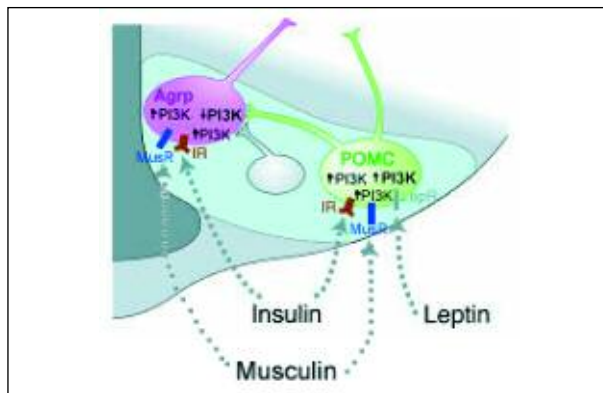


Figure 38. An alternative schematic representation of the modulation of arcuate nucleus neurons by leptin and insulin, and postulated modulation by *Musculin*. This figure is also a modification of Figure 29 and PI3K activity is depicted as a mediator and/or marker of neuronal activation and neuropeptide release in both AgRP (pink) and POMC (green) neurons. The effects of insulin on PI3K activity are direct in both neuronal subtypes, but the effects of leptin on PI3K activity in AgRP neurons require synaptic transmission from POMC or other (gray) inhibitory synaptic neurons. This figure proposes that the effects of *Musculin* on PI3K are also direct and that PI3K is increased in both neuronal subtypes. IR, insulin receptor, LepR, leptin receptor; MusR, *Musculin* receptor

creased ARC NPY mRNA observed in long-term exercise.

Future directions

Although much of the content of this manuscript is hypothetical, we suggest that a search for a protein with the characteristics described for *Musculin* may be worthwhile, since analogues of such a substance may be of therapeutic benefit in the management of the current global diabetes and obesity epidemic (552-560).

Acknowledgments

I dedicate this manuscript to Professors Seymour Reichlin, Albert Burger, and John Funder who have been lifetime mentors and friends. I am indebted to Professors Murray Esler, Ronald Lechan, and Larry Swanson for very helpful discussions, and for access to experimental data prior to publication (R.L.). This manuscript would never have seen the light of day were it not for the vehement insistence and unending patience of my dear friend, Professor Roberto Toni. To you Roberto, I say "Grazie tanto!"

References

1. Kishimoto T, Akira S, Taga T. Interleukin-6 and its receptor: a paradigm for cytokines. *Science* 1992; 258: 593-7.
2. Hibi M, Nakajima K, Hirano T. IL-6 cytokine family and signal transduction: a model of the cytokine system. *J Mol Med* 1996; 74: 1-12.
3. Alexander WS, Hilton DJ. The role of suppressor of cytokine signaling (SOCS) proteins in regulation of the immune response. *Annu Rev Immunol* 2004; 22: 503-29.
4. Pedersen BK, Steensberg A, Schjerling P. Muscle-derived interleukin 6: possible biological effects. *J Physiol (Lond)* 2001; 536: 329-37.
5. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB J* 2002; 16: 1335-47.
6. Hiscock N, Chan MHS, Bisucci T, Darby IA, Febbraio MA. Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. *FASEB J* 2004; 18: 992-4.
7. Steensberg A, van Hall G, Osada T, et al. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol (Lond)* 2000; 529: 237-42.
8. MacDonald C, Wojtaszewski JFP, Pedersen BK, Kiens B, Richter EA. Interleukin-6 release from human skeletal muscle during exercise: relation to AMPK activity. *J Appl Physiol* 2003; 2273-7.
9. Pedersen BK, Steensberg A, Fischer C, et al. Searching for the exercise factor: is IL-6 a candidate? *J Muscle Res Cell Motil* 2003; 24: 113-9.
10. Fischer CP, Plomgaard P, Hansen AK, Pilegaard H, Saltin B, Pedersen BK. Endurance training reduces the contraction-induced interleukin-6 mRNA expression in human skeletal muscle. *Am J Physiol* 2004; E1189-E1194.
11. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes* 2004; 53: 1643-8.
12. Febbraio MA, Pedersen BK. Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? *Exerc Sport Sci Reviews* 2005; 33: 114-9.
13. Kanemaki T, Kitade H, Kaibori M, et al. Interleukin-1 β and interleukin 6, but not tumor necrosis factor α , inhibit insulin-stimulated glycogen synthesis in rat hepatocytes. *Hepatology* 1998; 27: 1296-303.
14. Senn JJ, Klover PJ, Nowak IA, et al. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* 2003; 278: 13740-6.
15. Klover PJ, Clementi AH, Mooney RA. Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* 2005; 146: 3417-27.
16. Weigert C, Hennige AM, Brodbeck K, Häring HU, Schleicher ED. Interleukin-6 acts as insulin sensitizer on glycogen synthesis in human skeletal muscle cells by phosphorylation of Ser⁴⁷³ of Akt. *Am J Physiol* 2005; 289: E251-E257.

17. O'Doherty RM, Bracy DP, Osawa H, Wasserman DH, Granner DK. Rat skeletal muscle hexokinase II mRNA and activity are increased by a single bout of acute exercise. *Am J Physiol* 1994; 266: E171-E178.
18. Essig DA, Borger DR, Jackson DA. Induction of heme oxygenase-1 (HSP32) mRNA in skeletal muscle following contractions. *Am J Physiol* 1997; 272: C59-C67.
19. Koval JA, DeFronzo RA, O'Doherty RM, et al. Regulation of hexokinase II activity and expression in human muscle by moderate exercise. *Am J Physiol* 1998; 274: E304-E308.
20. Cortright RN, Zheng D, Jones JP, et al. Regulation of skeletal muscle UCP-2 and UCP-3 gene expression by exercise and denervation. *Am J Physiol* 1999; 276: E217-E221.
21. Pilegaard H, Ordway GA, Saltin B, Neufer PD. Transcriptional activation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol* 2000; 279: E806-E814.
22. Kraniou Y, Cameron-Smith D, Misso M, Collier G, Hargreaves M. Effects of exercise on GLUT-4 and glycogenin gene expression in human skeletal muscle. *J Appl Physiol* 2000; 88: 794-6.
23. Nielsen JN, Frosig C, Sajan MP, et al. Increased atypical PKC activity in endurance-trained human skeletal muscle. *Biochem Biophys Res Commun* 2003; 312: 1147-53.
24. Nordsborg N, Bangsbo J, Pilegaard H. Effect of high-intensity training on exercise-induced gene expression specific to ion homeostasis and metabolism. *J Appl Physiol* 2003; 95: 1201-6.
25. Jensen L, Pilegaard H, Neufer PD, Hellsten Y. Effect of acute exercise training on VEGF splice variants in human skeletal muscle. *Am J Physiol* 2004; 287: R397-R402.
26. Norrbom J, Sundberg CJ, Ameln H, Kraus WE, Jansson E, Gustafsson T. PGC-1 α mRNA expression is influenced by metabolic perturbation in exercising skeletal muscle. *J Appl Physiol* 2004; 96: 189-94.
27. Bickel CS, Slade J, Mahoney E, Haddad F, Dudley GA, Adams GR. Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. *J Appl Physiol* 2005; 98: 482-8.
28. Teran-Garcia M, Rankinen T, Koza RA, Rao DC, Bouchard C. Endurance training-induced changes in insulin sensitivity and gene expression. *Am J Physiol* 2005; 288: E1168-E1178.
29. Trinick J. Titin as a scaffold and spring. *Curr Biol* 1996; 6: 258-60.
30. Maruyama K. Connectin/titin, giant elastic protein of muscle. *FASEB J* 1997; 11: 341-5.
31. Mayans O, van der Ven PF, Wilm M, et al. Structural basis for activation of the titin kinase domain during myofibrillogenesis. *Nature* 1998; 395: 863-9.
32. Clark KA, McElhinny AS, Beckerle MC, Gregorio CC. Striated muscle cytoarchitecture: an intricate web of form and function. *Annu Rev Cell Dev Biol* 2002; 18: 637-706.
33. Tskhovrebova L, Trinick J. Titin: properties and family relationships. *Nat Rev Mol Cell Biol* 2003; 4: 679-89.
34. Lange S, Xiang F, Yakovenko A, et al. The kinase domain of titin controls muscle gene expression and protein turnover. *Science* 2005; 308: 1599-603.
35. Tskhovrebova L, Trinick J. Muscle disease: a giant feels the strain. *Nat Med* 2005; 11: 478-9.
36. Rothman JE. Mechanisms of intracellular protein transport. *Nature* 1994; 372: 55-64.
37. Südhof TC. The synaptic vesicle: a cascade of protein-protein interactions. *Nature* 1995; 375: 645-53.
38. Rothman JE. Lasker Basic Medical Research Award. The machinery and principles of vesicle transport in the cell. *Nat Med* 2002; 8: 1059-62.
39. Südhof TC. The synaptic vesicle cycle. *Annu Rev Neurosci* 2004; 27: 509-47.
40. Cain CC, Trimble WS, Lienhard GE. Members of the VAMP family of synaptic vesicle proteins are components of glucose transporter-containing vesicles from rat adipocytes. *J Biol Chem* 1992; 267: 11681-4.
41. Volchuk A, Mitsumoto Y, He L, et al. Expression of vesicle-associated membrane protein 2 (VAMP-2)/synaptobrevin II and cellubrevin in rat skeletal muscle and in a muscle cell line. *Biochem J* 1994; 304: 139-45.
42. Ralston E, Beushausen S, Ploug T. Expression of the synaptic vesicle proteins VAMPs/synaptobrevins 1 and 2 in non-neural tissues. *J Biol Chem* 1994; 269: 15403-6.
43. Sumitani S, Ramlal T, Liu Z, Klip A. Expression of syntaxin 4 in rat skeletal muscle and rat skeletal muscle cells in culture. *Biochem Biophys Res Commun* 1995; 213: 462-8.
44. Cheatham B, Volchuk A, Kahn CR, et al. Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins. *Proc Natl Acad Sci USA* 1996; 93: 15169-73.
45. Olson AL, Knight JB, Pessin JE. Syntaxin 4, VAMP2, and/or VAMP3/cellubrevin are functional target membrane and vesicle SNAP receptors for insulin-stimulated GLUT4 translocation in adipocytes. *Mol Cell Biol* 1997; 17: 2425-35.
46. Tellam JT, Macauley SL, McIntosh S, Hewish DR, Ward CW, James DE. Characterization of Munc-18c and Syntaxin-4 in 3T3-L1 adipocytes. Putative role in insulin-dependent movement of GLUT-4. *J Biol Chem* 1997; 272: 6179-86.
47. Zeng Q, Subramaniam VN, Wong SH, et al. A novel synaptobrevin/VAMP homologous protein (VAMP5) is increased during in vitro myogenesis and present in the plasma membrane. *Mol Cell Biol* 1998; 9: 2423-37.
48. Martin L, Shewan A, Millar CA, Gould GW, James DE. Vesicle-associated membrane protein 2 plays a specific role in the insulin-dependent trafficking of the facilitative glucose transporter GLUT4 in 3T3-L1 adipocytes. *J Biol Chem* 1998; 273: 1444-52.
49. Maier VH, Melvin DR, Lister CA, Chapman H, Gould GW, Murphy GJ. v- and t-SNARE protein expression in models of insulin resistance. Normalization of glycemia by rosiglitazone treatment corrects overexpression of cellubrevin, vesicle-associated membrane protein-2, and syntaxin-4 in skeletal muscle of Zucker diabetic fatty rats. *Diabetes* 2000; 49: 618-25.
50. Ueki K, Yballe CM, Brachmann SM, et al. Increased insulin sensitivity in mice lacking p85 β subunit of phosphoi-

- nositide 3-kinase. *Proc Natl Acad Sci USA* 2002; 99: 419-24.
51. Yamashita T, Hashiramoto A, Haluzik M, et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc Natl Acad Sci USA* 2003; 100: 3445-9.
 52. King DS, Dalsky GP, Staten MA, Clutter WE, van Houten DR, Holloszy JO. Insulin action and secretion in endurance-trained and untrained humans. *J Appl Physiol* 1987; 63: 2247-52.
 53. Mikines KJ, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *Am J Physiol* 1988; 254: E248-E259.
 54. Richter EA, Mikines KJ, Galbo H, Kiens ZB. Effect of exercise on insulin action in human skeletal muscle. *J Appl Physiol* 1989; 66: 876-85.
 55. Cartee G, Young D, Sleeper M, Zierath J, Wallberg-Henriksson H, Holloszy JO. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol* 1989; 256: E494-E499.
 56. Segal KR, Edano A, Abalos A, et al. Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *J Appl Physiol* 1991; 71: 2402-11.
 57. Mikines KJ. The influence of physical activity and inactivity on insulin action and secretion in man. *Acta Physiol Scand* 1992; 142, Suppl 609: 1-43.
 58. Dela F, Mikines KJ, Sonne B, Galbo H. Effect of training on interaction between insulin and exercise in human muscle. *J Appl Physiol* 1994; 76: 2386-93.
 59. Perseghin G, Price TB, Petersen KJ, et al. Increased glucose transport-phosphorylation and muscle glycogen synthesis after training in insulin resistant subjects. *N Engl J Med* 1996; 335: 1357-62.
 60. Goodyear LJ, Kahn BB. Exercise, glucose transport and insulin sensitivity. *Annu Rev Med* 1998; 49: 235-61.
 61. Zierath JR. Invited review: Exercise training-induced changes in insulin signaling in skeletal muscle. *J Appl Physiol* 2002; 93: 773-81.
 62. Henriksen EJ. Invited review: Effects of acute exercise and exercise training on insulin resistance. *J Appl Physiol* 2002; 93: 788-96.
 63. Nishida Y, Tokuyama K, Nagasaka S, et al. Effect of moderate exercise training on peripheral glucose effectiveness, insulin sensitivity, and endogenous glucose production in healthy humans estimated by a two-compartment-labeled minimal model. *Diabetes* 2004; 53: 315-20.
 64. Hayashi Y, Nagasaka S, Takahashi N, et al. A single bout of exercise at higher intensity enhances glucose effectiveness in sedentary men. *J Clin Endocrinol Metab* 2005; 90: 4035-40.
 65. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. *J Appl Physiol* 2005; 99: 338-43.
 66. Neuffer PD, Dohm GL. Exercise induces a transient increase in transcription of the GLUT-4 gene in skeletal muscle. *Am J Physiol* 1993; 265: C1597-C1603.
 67. Roy D, Marette A. Exercise induces the translocation of GLUT4 to transverse tubules from an intracellular pool in rat skeletal muscle. *Biochem Biophys Res Commun* 1996; 223: 147-52.
 68. Hayashi T, Wojtaszewski JF, Goodyear LJ. Exercise regulation of glucose transport in skeletal muscle. *Am J Physiol* 1997; 273: E1039-E1051.
 69. Hansen PA, Nolte LA, Chen MM, Holloszy JO. Increased GLUT-4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise. *J Appl Physiol* 1998; 85: 1218-12.
 70. Kim YB, Inoue T, Nakajima R, Shirai-Morishita Y, Tokuyama K, Suzuki M. Effect of long-term exercise on gene expression of insulin signaling pathway intermediates. *Biochem Biophys Res Commun* 1999; 254: 720-7.
 71. Kuo CH, Browning KS, Ivy J. Regulation of GLUT4 protein expression and glycogen storage after prolonged exercise. *Acta Physiol Scand* 1999; 165: 193-201.
 72. Kranioy Y, Cameron-Smith D, Misso M, Collier G, Hargreaves M. Effects of exercise on GLUT-4 and glycogenin gene expression in human skeletal muscle. *J Appl Physiol* 2000; 88: 794-6.
 73. Dugaard JR, Nielsen JN, Kristiansen S, Andersen JL, Hargreaves M, Richter EA. Fiber type-specific expression of GLUT4 in human skeletal muscle: influence of exercise training. *Diabetes* 2000; 49: 1092-5.
 74. MacLean PS, Zheng D, Jones JP, Olson AL, Dohm GL. Exercise-induced transcription of the muscle glucose transporter (GLUT4) gene. *Biochem Biophys Res Commun* 2002; 292: 409-14.
 75. Jessen N, Pold R, Buhl ES, Jensen LS, Schmitz O, Lund S. Effects of AICAR and exercise on insulin-stimulated glucose uptake, signaling, and GLUT-4 content in rat muscles. *J Appl Physiol* 2003; 94: 1373-9.
 76. Arkininstall MJ, Tunstall RJ, Cameron-Smith D, Hawley JA. Regulation of metabolic genes in skeletal muscle by short-term exercise and diet manipulation. *Am J Physiol* 2004; 287: E25-E31.
 77. Holtén MK, Zacho M, Gaster M, Joel C, Wojtaszewski JF, Dela F. Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes* 2004; 53: 294-305.
 78. Lohmann D, Liebold F, Heilmann W, Senger H, Pohl A. Diminished insulin response in highly trained athletes. *Metabolism* 1978; 27: 521-4.
 79. Järhult J, Holst J. The role of adrenergic innervation to the pancreatic islets in the control of insulin release during exercise in man. *Pflüg Arch Eur J Physiol* 1979; 383: 41-5.
 80. Wirth A, Diehm C, Mayer H et al 1981. Plasma C-peptide and insulin in trained and untrained subjects. *J Appl Physiol* 1981; 50: 71-7.
 81. Richard D, LeBlanc J. Pancreatic insulin response in relation to exercise training. *Can J Physiol Pharmacol* 1983; 63: 1194-7.
 82. Ahrén B, Veith RC, Taborsky GJ Jr. Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 1) Effects on basal release of insulin and glucagon. *Endocrinology* 1987; 121: 323-31.
 83. Scheurink AJW, Steffens AB, Benthem B. Central and peripheral adrenoceptors affect glucose, free fatty acids, and

- insulin in exercising rats. *Am J Physiol* 1988; 255: R547-R556.
84. Wasserman DH, Williams PE, Lacy DB, Goldstein RE, Cherrington AD. Exercise-induced fall in insulin and hepatic carbohydrate metabolism during muscular work. *Am J Physiol* 1989; 256: E500-E509.
85. Scheurink AJW, Steffens AB, Bouritius H, et al. Sympathoadrenal influence on glucose, FFA, and insulin levels in exercising rats. *Am J Physiol* 1989; 256: R161-R168.
86. Wasserman DH, Lacy DB, Goldstein RE, Williams PE, Cherrington AD. Exercise-induced fall in insulin and increase in fat metabolism during prolonged muscular work. *Diabetes* 1989; 38: 484-90.
87. Pestell RG, Ward GM, Galvin P, Best JD, Alford FP. Impaired glucose tolerance after endurance exercise is associated with reduced insulin secretion rather than altered insulin sensitivity. *Metabolism* 1993; 42: 277-82.
88. Zinker BA, Mohr T, Kelly P, Namdaran K, Bracy DP, Wasserman DH. Exercise-induced fall in insulin: mechanism of action at the liver and effects on muscle glucose metabolism. *Am J Physiol* 1994; E683-E689.
89. Houwing H, Fränkel KM, Strubbe JH, van Suylichem PTR, Steffens AB. Role of the sympathoadrenal system in exercise-induced inhibition of insulin secretion. Effects of islet transplantation. *Diabetes* 1995; 44: 565-71.
90. Engdahl JH, Veldhuis JD, Farrell PA. Altered pulsatile insulin secretion associated with endurance training. *J Appl Physiol* 1995; 79: 1977-85.
91. Lavoie C, Chiasson JL, Ducros F, Bourque J, Langelier H. Glucose metabolism during exercise in man: the role of insulin and glucagon in the regulation of hepatic glucose production and gluconeogenesis. *Can J Physiol Pharmacol* 1997; 75: 26-35.
92. Kishimoto H, Taniguchi A, Fukushima M, et al. Effect of short-term low-intensity exercise on insulin sensitivity, insulin secretion, and glucose and lipid metabolism in non-obese Japanese type 2 diabetic patients. *Horm Metab Res* 2002; 34: 27-31.
93. Böttger I, Schlein E, Faloona GR, Knochel JP, Unger RH. The effect of exercise on glucagon secretion. *J Clin Endocrinol Metab* 1972; 35: 117-25.
94. Luyckx AS, Lefebvre PJ. Mechanisms involved in the exercise-induced increase in glucagon secretion in rats. *Diabetes* 1974; 23: 81-93.
95. Galbo H, Holst JJ, Christensen NJ. Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. *J Appl Physiol* 1975; 38: 70-6.
96. Vranic M, Kawamori R, Pek S, Kovacevic N, Wrenshall GA. The essentiality of insulin and the role of glucagon in regulating glucose utilization and production during strenuous exercise in dogs. *J Clin Invest* 1976; 57: 245-55.
97. Galbo H, Christensen NJ, Holst JJ. Catecholamines and pancreatic hormones during autonomic blockade in exercising man. *Acta Physiol Scand* 1977; 101: 428-37.
98. Galbo H, Richter EA, Hilsted J, Holst JJ, Christensen NJ, Henriksson J. Hormonal regulation during prolonged exercise. *Ann NY Acad Sci* 1977; 301: 72-80.
99. Luyckx AS, Pirnay F, Lefebvre PJ. Effect of glucose on plasma glucagon and free fatty acids during prolonged exercise. *Eur J Appl Physiol* 1978; 39: 53-61.
100. Sotsky MJ, Shilo S, Shamooh H. Regulation of counter-regulatory hormone secretion in man during exercise and hypoglycemia. *J Clin Endocrinol Metab* 1989; 68: 9-16.
101. Brockman RP. Effect of somatostatin on plasma glucagon and insulin, and glucose turnover in exercising sheep. *J Appl Physiol* 1979; 47: 273-8.
102. Brockman RP, Halvorson R. Glucose, glucagon, and insulin during adrenergic blockade in exercising sheep. *J Appl Physiol* 1982; 52: 315-9.
103. Coupland RE. The innervation of the pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. *J Anat* 1958; 92: 143-9.
104. Cegrell L. Adrenergic nerves and monoamine-containing cells in the mammalian endocrine pancreas. *Acta Physiol Scand* 1968; 314: 17-23.
105. Ahrén B, Ericson LE, Lundquist I, Lorén I, Sundler F. Adrenergic innervation of pancreatic islets and modulation of insulin secretion by the sympatho-adrenal system. *Cell Tissue Res* 1981; 216: 15-30.
106. Berthoud HR, Powley TL. Identification of vagal preganglionic neurons that mediate cephalic phase insulin response. *Am J Physiol* 1990; 258: R523-R530.
107. Loewy AD, Franklin MF, Haxhiu MA. CNS monoamine cell groups projecting to pancreatic vagal motor neurons: a transneuronal labeling study using pseudorabies virus. *Brain Res* 1994; 638: 248-60.
108. Jansen AS, Hoffman JL, Loewy AD. CNS sites involved in sympathetic and parasympathetic control of the pancreas: a viral tracing study. *Brain Res* 1997; 766: 29-38.
109. Streefland C, Maes FW, Bohus B. Autonomic brainstem projections to the pancreas: a retrograde transneuronal viral tracing study in the rat. *J Auton Nerv Syst* 1998; 74: 71-81.
110. Buijs RM, Chun SJ, Nijijima A, Romijn HJ, Nagai K. Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. *J Comp Neurol* 2001; 431: 405-23.
111. Kiba T. Relationships between the autonomic nervous system and the pancreas including regulation of regeneration and apoptosis: recent developments. *Pancreas* 2004; 29: 51-8.
112. Frohman LA, Ezdinli EZ, Javid R. Effect of vagotomy and vagal stimulation on insulin stimulation. *Diabetes* 1967; 16: 443-8.
113. Porte D Jr, Girardier L, Seydoux J, Kanazawa Y, Posternak J. Neural regulation of insulin secretion in the dog. *J Clin Invest* 1973; 52: 210-4.
114. Marliss EB, Girardier L, Seydoux J, et al. Glucagon release induced by pancreatic nerve stimulation in the dog. *J Clin Invest* 1973; 52: 1246-59.
115. Harvey WD, Faloona GR, Unger RH. The effect of adrenergic blockade on exercise-induced hyperglucagonemia. *Endocrinology* 1974; 94: 1254-8.

116. Kaneto A, Miki E, Kosaka K. Effects of vagal stimulation on glucagon and insulin secretion. *Endocrinology* 1974; 95: 1005-10.
117. Woods SC, Porte D Jr. Neural control of the endocrine pancreas. *Physiol Rev* 1974; 54: 596-619.
118. Samols E, Weir GC. Adrenergic modulation of A, B, and D cells. α -adrenergic suppression and β -adrenergic stimulation of somatostatin secretion, α -adrenergic stimulation of glucagon secretion in the perfused dog pancreas. *J Clin Invest* 1979; 230-8.
119. Ahrén B, Taborsky GJ Jr. The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog. *Endocrinology* 1986; 118: 1551-7.
120. Ahrén B, Taborsky GJ Jr. Effects of pancreatic noradrenaline infusion on basal and stimulated islet hormone secretion in the dog. *Acta Physiol Scand* 1988; 132 (2): 143-50.
121. Miller RE. Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the Islets of Langerhans. *Endocr Rev* 1981; 2: 471-94.
122. Ahrén B. Autonomic regulation of islet hormone secretion-implications for health and disease. *Diabetologia* 2000; 43: 393-410.
123. Coker RH, Koyama Y, Lacy DB, Williams PE, Thèaume N, Wasserman DH. Pancreatic innervation is not essential for exercise-induced changes in glucagon and insulin or glucose kinetics. *Am J Physiol* 1999; E1122-E1129.
124. Buschard K, Aaen K, Horn T, van Damme J, Bendtzen K. Interleukin 6: a functional and structural in vitro modulator of β -cells from islets of Langerhans. *Autoimmunity* 1990; 5: 185-94.
125. Robinson BF, Epstein SE, Beiser GD, Braunwald E. Control of heart rate by the autonomic nervous system. Studies in man on the interrelation between baroreceptor mechanisms and exercise. *Circ Res* 1966; 19: 400-11.
126. Freyschuss U. Elicitation of heart rate and blood pressure increase on muscle contraction. *J Appl Physiol* 1970; 28: 758-61.
127. Duncan JJ, Farr JE, Upton SJ, Hagan RD, Oglesby ME, Blair SN. The effects of aerobic exercise on plasma catecholamines and blood pressure in patients with mild hypertension. *JAMA* 1985; 254: 2609-13.
128. Kiyonaga A, Arakawa K, Tanaka H, Shindo M. Blood pressure and hormonal responses to aerobic exercise. *Hypertension* 1985; 7: 125-31.
129. Jennings G, Nelson L, Nestel P, et al. The effects of changes in physical activity on major cardiovascular risk factors, hemodynamics, sympathetic function, and glucose utilization in man: a controlled study of four levels of activity. *Circulation* 1986; 73: 30-40.
130. Hasking GJ, Esler MD, Jennings GL, Dewar E, Lambert G. Norepinephrine spillover to plasma during steady-state supine bicycle exercise. Comparison of patients with congestive cardiac failure and normal subjects. *Circulation* 1988; 78: 516-21.
131. Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation. Source, fate and functions. *Physiol Rev* 1990; 70: 963-85.
132. Meredith IT, Friberg P, Jennings GL, et al. Exercise training lowers resting renal but not cardiac sympathetic activity in humans. *Hypertension* 1991; 18: 575-82.
133. Mazzeo RS, Rajkumar C, Jennings G, Esler M. Norepinephrine spillover at rest and during submaximal exercise in young and old subjects. *J Appl Physiol* 1997; 82: 1869-74.
134. Kohno K, Matsuoka H, Takenaka K, et al. Depressor effect by exercise training is associated with amelioration of hyperinsulinemia and sympathetic overactivity. *Intern Med* 2000; 39: 1013-9.
135. Himsworth HP. The mechanism of diabetes mellitus. I. *Lancet* 1939; 234: 1-6.
136. Himsworth HP. The mechanism of diabetes mellitus. II. The control of the blood-sugar level. *Lancet* 1939; 234: 65-8.
137. Himsworth HP. The mechanism of diabetes mellitus. II. The control of the blood-sugar level (contd). *Lancet* 1939; 234: 118-22.
138. Himsworth HP. The mechanism of diabetes mellitus. III. Human diabetes mellitus. *Lancet* 1939; 234: 171-6.
139. Bornstein J, Lawrence DD. Plasma insulin in human diabetes mellitus. *BMJ* 1951; 2: 1541-4.
140. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. *J Clin Invest* 1960; 39: 1157-75.
141. Ginsberg H, Kimmerling G, Olesfsky JM, Reaven GM. Demonstration of insulin resistance in untreated adult onset diabetic subjects with fasting hyperglycemia. *J Clin Invest* 1975; 55: 454-61.
142. DeFronzo RA. Lilly Lecture 1987. The triumvirate: β -cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988; 37: 667-87.
143. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; 37: 1595-607.
144. Lillioja S, Mott DM, Howard BE, et al. Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. *N Engl J Med* 1988; 318: 467-72.
145. Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med* 1990; 113: 909-12.
146. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* 1992; 340: 925-9.
147. Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 1993; 329: 1988-92.
148. Reaven GM. Why syndrome X? From Harold Himsworth to the Insulin Resistance Syndrome. *Cell Metab* 2005; 9-14.
149. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle: its role in insulin sensitivity and

- the metabolic disturbances of diabetes mellitus. *Lancet* 1963; 1: 785-789.
150. Rothman DL, Shulman RG, Shulman GI. ³¹P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. *J Clin Invest* 1992; 89: 1069-75.
 151. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science* 1993; 259: 87-91.
 152. Hotamisligil GS, Peraldi P, Spiegelman BM. The molecular link between obesity and diabetes. *Curr Opin Endocrinol Diab* 1996; 3:16-23.
 153. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF- α function. *Nature* 1997; 389:610-4.
 154. Brüning JC, Michael MD, Winnay JN, et al. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 1998; 2: 559-69.
 155. Dresner A, Laurent D, Marcucci M, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 1999; 103: 253-9.
 156. Cline GW, Petersen KF, Krssak M, et al. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med* 1999; 341:240-246.
 157. Perseghin G, Scifo P, De Cobelli F, et al. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans. A ¹H-¹³C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic patients. *Diabetes* 1999; 48: 1600-6.
 158. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000; 106: 171-6.
 159. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 2000; 49: 677-83.
 160. Zisman A, Peroni OD, Abel ED, et al. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med* 2000; 6: 924-8.
 161. Saltiel AR. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. *Cell* 2001; 104: 517-29.
 162. Minokoshi Y, Kahn CR, Kahn BB. Tissue-specific ablation of the GLUT4 glucose transporter or the insulin receptor challenges assumptions about insulin action and glucose homeostasis. *J Biol Chem* 2003; 278: 33609-12.
 163. Norris AW, Chen L, Fisher SJ, et al. Muscle-specific PPAR γ -deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones. *J Clin Invest* 2003; 112: 608-18.
 164. Hevener AL, He W, Barak Y, et al. Muscle-specific Pparg deletion causes insulin resistance. *Nat Med* 2003; 1491-7.
 165. Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 2003; 300: 1140-2.
 166. Muoio DM, Newgard CB. Insulin resistance takes a trip through the ER. *Science* 2004; 306: 425-6.
 167. Özcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004; 306: 457-61.
 168. Oshikawa J, Otsu K, Toya Y, et al. Insulin resistance in skeletal muscles of caveolin-3-null mice. *Proc Natl Acad Sci USA* 2004; 12670-5.
 169. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. *Science* 2005; 307: 384-7.
 170. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; 115: 1111-9.
 171. Pederson O, Bak JF, Andersen S, et al. Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. *Diabetes* 1990; 39: 865-70.
 172. Garvey WT, Maianu L, Huecksteadt TP, Birnbaum MJ, Molina MJ, Ciaraldi TP. Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. *J Clin Invest* 1991; 87: 1072-81.
 173. Garvey WT, Maianu L, Hancock JA, Golichowski AM, Baron A. Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. *Diabetes* 1992; 41: 465-75.
 174. Garvey WT, Maianu L, Zhu J-H, Brechtel-Hook G, Wallace P, Baron AD. Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *J Clin Invest* 1998; 101: 2377-86.
 175. Shepherd PR, Kahn BB. Glucose transporters and insulin action-implications for insulin resistance and diabetes mellitus. *N Engl J Med* 1999; 341: 248-57.
 176. Bonner-Weir S. Regulation of pancreatic β -cell mass in vivo. *Recent Prog Horm Res* 1994; 49: 91-104.
 177. Scaglia L, Cahill CJ, Finegood DT, Bonner-Weir S. Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. *Endocrinology* 1997; 138: 1736-41.
 178. Bonner-Weir S. Islet growth and development in the adult. *J Mol Endocrinol* 2000; 24: 297-302.
 179. Laybutt DR, Weir GC, Kaneto H, et al. Overexpression of c-Myc in β -cells of transgenic mice causes proliferation and apoptosis, downregulation of insulin gene expression, and diabetes. *Diabetes* 2002; 51: 1793-804.
 180. Svenstrup K, Skau M, Pakkenberg B, Buschard K, Bock T. Postnatal development of β -cells in rats. Proposed explanatory model. *APMIS* 2002; 110: 372-8.
 181. Engler D, Scanlon MF, Jackson IMD. Thyrotropin-releasing hormone in the systemic circulation of the neonatal rat is derived from the pancreas and other extraneural tissues. *J Clin Invest* 1981; 67: 800-8.
 182. Aratan-Spire S, Wolf B, Portha B, Bailbé D, Czernichow P. Streptozotocin treatment at birth induces a parallel de-

- pletion of thyrotropin-releasing hormone and insulin in the rat pancreas during development. *Endocrinology* 1984; 114: 369-73.
183. Leduque P, Aratan-Spire S, Czernichow P, Dubois P-M. Ontogenesis of thyrotropin-releasing hormone in the human fetal pancreas. A combined radioimmunological and immunocytochemical study. *J Clin Invest* 1986; 78: 1028-34.
 184. Leduque P, Aratan-Spire S, Scharfmann R, Basmaciogullari A, Czernichow P, Dubois P-M. Coexistence of thyrotropin-releasing hormone and insulin in cultured fetal rat islets: a light and electron microscopic immunocytochemical study during islet neof ormation. *Biol Cell* 1989; 66: 291-6.
 185. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic β -cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004; 429: 41-6.
 186. Georgia S, Bhushan A. β cell replication is the primary mechanism for maintaining postnatal β cell mass. *J Clin Invest* 2004; 114: 963-8.
 187. Sherr CJ. Cancer cell cycles. *Science* 1996; 274: 1672-7.
 188. Morgan DO. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu Rev Cell Dev Biol* 1997; 13: 261-91.
 189. Woo RA, Poon RY. Cyclin-dependent kinases and S phase control in mammalian cells. *Cell Cycle* 2003; 2: 316-24.
 190. Murray AW. Recycling the cell cycle: cyclins revisited. *Cell* 2004; 116: 221-34.
 191. Sherr CJ, Roberts JM. Living with or without cyclins and cyclin-dependent kinases. *Genes Dev* 2004; 18: 2699-711.
 192. Deshpande A, Sicinski P, Hinds PW. Cyclins and cdk in development and cancer: a perspective. *Oncogene* 2005; 24: 2909-15.
 193. Kushner JA, Ciemerych MA, Sicinska E, et al. Cyclins D2 and D1 are essential for postnatal pancreatic β -cell growth. *Mol Cell Biol* 2005; 25: 3752-62.
 194. Rane SG, Dubus P, Mettus RV, et al. Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in β -islet cell hyperplasia. *Nat Genet* 1999; 22: 44-52.
 195. Brüning JC, Winnay J, Bonner-Weir S, Taylor SI, Accili D, Kahn CR. Development of a novel polygenic model of NIDDM in mice heterozygous for *IR* and *IRS-1* null alleles. *Cell* 1997; 88: 561-72.
 196. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. β -cell deficit and increased β -cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003; 52: 102-10.
 197. Sicinski P, Donaher JL, Geng Y, et al. Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. *Nature* 1996; 384: 470-4.
 198. Flier SN, Kulkarni RN, Kahn CR. Evidence for a circulating islet cell growth factor in insulin-resistant states. *Proc Natl Acad Sci USA* 2001; 98: 7475-7480.
 199. Michael MD, Kulkarni RN, Postic C, et al. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 2000; 6: 87-97.
 200. Sutton J, Lazarus L. Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J Appl Physiol* 1976; 523-7.
 201. Bunt JC, Boileau RA, Bahr JM, Nelson RA. Sex and training differences in human growth hormone levels during prolonged exercise. *J Appl Physiol* 1986; 61: 1796-801.
 202. Felsing NE, Brasel JA, Cooper DM. Effect of low and high-intensity exercise on circulating growth hormone in men. *J Clin Endocrinol Metab* 1992; 75: 157-62.
 203. Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr Rev* 1998; 19: 717-97.
 204. Pritzlaff CJ, Wideman L, Wideman JY, et al. Impact of acute exercise intensity on pulsatile growth hormone release in men. *J Appl Physiol* 1999; 87: 498-504.
 205. Godfrey RJ, Madgwick Z, Whyte GP. The exercise-induced growth hormone response in athletes. *Sports Med* 2003; 33: 599-613.
 206. Jorgensen JO, Krag M, Kanaley J, et al. Exercise, hormones, and body temperature. Regulation and action of GH during exercise. *J Endocrinol Invest* 2003; 26: 838-42.
 207. Weltman A, Wideman L, Weltman JY, Veldhuis JD. Neuroendocrine control of GH release during acute aerobic exercise. *J Endocrinol Invest* 2003; 26: 843-850.
 208. Few JD. Effect of exercise on the secretion and metabolism of cortisol in man. *J Endocrinol* 1974; 62: 341-53.
 209. Brandenberger G, Follenius M. Influence of timing and intensity of muscular exercise on temporal patterns of plasma cortisol levels. *J Clin Endocrinol Metab* 1975; 40: 845-9.
 210. Gawel MJ, Park DM, Alagband-Zadeh J, Rose FC. Exercise and hormonal secretion. *Postgrad Med J* 1979; 55: 373-6.
 211. Alexander SL, Irvine CH, Ellis MJ, Donald RA. The effect of acute exercise on the secretion of corticotropin-releasing factor, arginine vasopressin, and adrenocorticotropin as measured in pituitary venous blood from the horse. *Endocrinology* 1991; 128: 65-72.
 212. Kanaley JA, Weltman JY, Pieper KS, Weltman A, Hartman ML. Cortisol and growth hormone responses to exercise at different times of day. *J Clin Endocrinol Metab* 2001; 2881-9.
 213. Hale RW, Kosasa T, Krieger J, Pepper S. A marathon: the immediate effect on female runners' luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, and cortisol levels. *Am J Obstet Gynecol* 1983; 146: 550-6.
 214. Smallridge RC, Whorton NE, Burman KD, Ferguson EW. Effects of exercise and physical fitness on the pituitary-thyroid axis and on prolactin secretion in male runners. *Metabolism* 1985; 34: 949-54.
 215. Chang FE, Dodds WG, Sullivan M, Kim MH, Malarkey WB. The acute effects of exercise on prolactin and growth hormone secretion: comparison between sedentary women and women runners with normal and abnormal menstrual cycles. *J Clin Endocrinol Metab* 1986; 62: 551-6.
 216. Farrell PA, Gustafson AB, Garthwaite TL, Kalkhoff RK, Cowley AW Jr, Morgan WP. Influence of endogenous

- opioids on the response of selected hormones to exercise in humans. *J Appl Physiol* 1986; 61: 1051-7.
217. Odink J, van der Beek EJ, van den Berg H, Bogaards JJ, Thissen JT. Effect of work load on free and sulfate-conjugated plasma catecholamines, prolactin, and cortisol. *Int J Sports Med* 1986; 7: 352-7.
218. Brammert M, Hökfelt B. The influence of naloxone on exercise-induced increase in plasma pituitary hormones and the subjectively experienced level of exhaustion in healthy males. *Acta Endocrinol (Copenh)* 1987; 115: 125-30.
219. Keizer HA, Kuipers H, de Haan J, et al. Effect of a 3-month endurance training program on metabolic and multiple hormonal responses to exercise. *Int J Sports Med* 1987; 8 Suppl 3: 154-60.
220. Luger A, Watschinger B, Deuster P, Svoboda T, Clodi M, Chrousos GP. Plasma growth hormone and prolactin responses to graded levels of acute exercise and to a lactate infusion. *Neuroendocrinology* 1992; 56: 112-7.
221. Boisvert P, Brisson GR, Peronnet F. Effect of plasma prolactin on sweat rate and sweat composition during exercise in men. *Am J Physiol* 1993; 264: F816-F820.
222. Struder HK, Hollmann W, Platen P, Wostmann R, Ferrauti A, Weber K. Effect of exercise intensity on free tryptophan to branched-chain amino acids ratio and plasma prolactin during endurance exercise. *Can J Appl Physiol* 1997; 22: 280-91.
223. van der Pompe G, Bernardis N, Kavelaars A, Heijnen C. An exploratory study into the effect of exhausting bicycle exercise on endocrine and immune responses in post-menopausal women: relationships between vigour and plasma cortisol concentrations and lymphocyte proliferation following exercise. *Int J Sports Med* 2001; 22: 447-53.
224. Cumming DC, Brunsting LA, Stich G, Ries AL, Rebar RW. Reproductive hormone increases in response to acute exercise in men. *Med Sci Sports Exerc* 1986; 18: 369-73.
225. Hakkinen K, Pakarinen A, Alen M, Kauhanen H, Komi PV. Neuromuscular and hormonal adaptations in athletes to strength training in two years. *J Appl Physiol* 1988; 65: 2406-12.
226. McColl EM, Wheeler GD, Gomes P, Bhambhani Y, Cumming DC. The effects of acute exercise on pulsatile LH release in high-mileage male runners. *Clin Endocrinol* 1989; 31: 617-21.
227. Pannier JL, Calders P, Eechaute W. Effect of adrenergic receptor blockade on plasma testosterone response to exercise in conscious dogs. *Arch Int Physiol Biochim Biophys* 1994; 102: 195-8.
228. Pieper DR, Ali HY, Benson LL, Shows MD, Loboocki CA, Subramanian MG. Voluntary exercise increases gonadotropin secretion in male golden hamsters. *Am J Physiol* 1995; 269: R179-R185.
229. Tremblay MS, Copeland JL, Van Helder W. Effect of training status and exercise mode on endogenous steroid hormones in men. *J Appl Physiol* 2004; 96: 531-9.
230. Tremblay MS, Copeland JL, Van Helder W. Influence of exercise duration on post-exercise steroid hormone responses in trained males. *Eur J Appl Physiol* 2005; 94: 505-13.
231. Bonen A, Ling WY, MacIntyre KP, Neil R, McGrail JC, Belcastro AN. Effects of exercise on the serum concentrations of FSH, LH, progesterone, and estradiol. *Eur J Appl Physiol* 1979; 43: 15-23.
232. Jurkowski JE, Jones NL, Walker C, Younglai EV, Sutton JR. Ovarian hormonal responses to exercise. *J Appl Physiol* 1978; 44: 109-14.
233. Keizer HA, Poortman J, Bunnik GSJ. Influence of physical exercise on sex-hormone metabolism. *J Appl Physiol* 1980; 45: 765-9.
234. Bonen A, Keizer HA. Pituitary, ovarian, and adrenal hormone responses to marathon running. *Int J Sports Med* 1987; 8 Suppl 3: 161-7.
235. Bonen A, Haynes FW, Graham TE. Substrates and hormonal responses to exercise in women using oral contraceptives. *J Appl Physiol* 1991; 70: 1917-27.
236. Consitt LA, Copeland JL, Tremblay MS. Endogenous anabolic hormone responses to endurance versus resistance exercise and training in women. *Sports Med* 2002; 32: 1-22.
237. Galbo H, Hummer L, Peterson IB, Christensen NJ, Bie N. Thyroid and testicular hormone responses to graded and prolonged exercise in man. *Eur J Appl Physiol* 1977; 36: 101-6.
238. Wirth A, Holm G, Lindstedt G, Lundberg PA, Björntorp P. Thyroid hormones and lipolysis in physically trained rats. *Metabolism* 1981; 30: 237-41.
239. Krotkiewski M, Sjöström L, Sullivan L, et al. The effect of acute and chronic exercise on thyroid hormones in obesity. *Acta Med Scand* 1984; 216: 269-75.
240. Hashimoto T, Migita S, Matsubara F. Response of thyrotropin, prolactin and free thyroid hormones to graded exercise in normal male subjects. *Endocrinol Jpn* 1986; 33: 735-41.
241. Sander M, Rocker L. Influence of marathon running on thyroid hormones. *Int J Sports Med* 1988; 9: 123-6.
242. Katzeff HL, Bovbjerg D, Mark DA. Exercise regulation of triiodothyronine metabolism. *Am J Physiol* 1988; 255: E824-E828.
243. Hackney AC, Gullledge T. Thyroid hormone responses during an 8-hour period following aerobic and anaerobic exercise. *Physiol Res* 1994; 43: 1-5.
244. Forner MA, Barriga C, Ortega E. Exercise-induced stimulation of murine macrophage phagocytosis may be mediated by thyroxine. *J Appl Physiol* 1996; 80: 899-903.
245. Ortega E, Rodriguez MJ, Barriga C, Forner MA. Corticosterone, prolactin and thyroid hormones as hormonal mediators of the stimulated phagocytic capacity of peritoneal macrophages after high-intensity exercise. *Int J Sports Med* 1996; 17: 149-55.
246. Crews EL III, Fuge KW, Oscari LB, Holloszy JO, Shank RE. Weight, food intake, and body composition: effects of exercise and of protein deficiency. *Am J Physiol* 1969; 216: 359-63.
247. Oscari LB, Holloszy JO. Effects of weight changes produced by exercise, food restriction, or overeating on body composition. *J Clin Invest* 1969; 48: 2124-8.
248. King NA, Burley VJ, Blundell JE. Exercise-induced sup-

- pression of appetite: effects on food intake and implications for energy balance. *Eur J Clin Nutr* 1994; 48: 715-24.
249. King NA, Snell L, Smith RD, Blundell JE. Effects of short-term exercise on appetite responses in unrestrained females. *Eur J Clin Nutr* 1996; 50: 663-7.
 250. King NA, Lluch A, Stubbs RJ, Blundell JE. High dose exercise does not increase hunger or energy intake in free living males. *Eur J Clin Nutr* 1997; 51: 478-83.
 251. King NA, Tremblay A, Blundell JE. Effects of exercise on appetite control: implications for energy balance. *Med Sci Sports Exerc* 1997; 29: 1076-89.
 252. Blundell JE, King NA. Effects of exercise on appetite control: loose coupling between energy expenditure and energy intake. *Int J Obes Relat Metab Disord* 1998; Suppl 2: S22-S29.
 253. Stubbs RJ, Sepp A, Hughes DA, et al. The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. *Eur J Clin Nutr* 2002; 56: 129-40.
 254. Stubbs RJ, Sepp A, Hughes DA, et al. The effect of graded levels of exercise on energy intake and balance in free-living women. *Int J Obes Relat Metab Disord* 2002; 26: 866-9.
 255. Frohman LA, Kineman RD, Kamegai J, et al. Secretagogues and the somatotrope: signaling and proliferation. *Recent Prog Horm Res* 2000; 55: 269-90.
 256. Veldhuis JD, Anderson SM, Shah N, et al. Neurophysiological regulation and target-tissue impact of the pulsatile mode of growth hormone secretion in the human. *Growth Horm IGF Res* 2001; 11 Suppl A: S25-S37.
 257. Kojima M, Hosoda H, Date Y, et al. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; 141: 656-60.
 258. Schmidt A, Maier C, Schaller G, et al. Acute exercise has no effect on ghrelin plasma concentrations. *Horm Metab Res* 2004; 36: 174-7.
 259. Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L, Hintz RL. Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science* 1981; 212: 1279-81.
 260. Yamashita S, Melmed S. Insulin-like growth factor 1 action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* 1986; 118: 176-82.
 261. Yamashita S, Melmed S. Insulinlike growth factor 1 regulation of growth hormone gene transcription in primary rat pituitary cells. *J Clin Invest* 1987; 79: 449-52.
 262. Niiori-Onishi A, Iwasaki Y, Mutsuga N, Oiso Y, Inoue K, Saito H. Molecular mechanisms of the negative effect of insulin-like growth factor-1 on growth hormone gene expression in MtT/S somatotroph cells. *Endocrinology* 1999; 140: 344-9.
 263. Rivier J, Spiess J, Thorner M, Vale W. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature* 1982; 300: 276-8.
 264. Guillemin R, Brazeau P, Böhlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* 1982; 218: 585-7.
 265. Bloch B, Brazeau P, Ling N, et al. Immunohistochemical detection of growth hormone-releasing factor in brain. *Nature* 1983; 301: 607-8.
 266. Frohman LA, Downs TR, Clarke IJ, Thomas GB. Measurement of growth hormone-releasing hormone and somatostatin in hypothalamic-portal plasma of unanesthetized sheep. Spontaneous secretion and response to insulin-induced hypoglycemia. *J Clin Invest* 1990; 86: 17-24.
 267. Mayo KE. Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Mol Endocrinol* 1992; 6: 1734-44.
 268. Bilezikjian LM, Vale WW. Stimulation of adenosine 3',5'-monophosphate production by growth hormone-releasing factor and its inhibition by somatostatin in anterior pituitary cells *in vitro*. *Endocrinology* 1983; 113: 1726-31.
 269. Holl RW, Thorner MO, Leong DA. Intracellular calcium concentration and growth hormone secretion in individual somatotropes: effects of growth hormone-releasing factor and somatostatin. *Endocrinology* 1988; 122: 2927-32.
 270. Lussier BT, French MB, Moor BC, Kraicer J. Free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and growth hormone release from purified rat somatotrophs.I.GH-releasing factor-induced Ca^{2+} influx raises $[Ca^{2+}]_i$. *Endocrinology* 1991; 128: 570-82.
 271. Barinaga M, Yamonoto G, Rivier C, Vale W, Evans R, Rosenfeld MG. Transcriptional regulation of growth hormone gene expression by growth hormone-releasing factor. *Nature* 1983; 306: 84-5.
 272. Brazeau P, Vale W, Burgus R, et al. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 1973; 179: 77-9.
 273. Alpert L, Brawer JR, Patel YC, Reichlin S. Somatostatinergic neurons in anterior hypothalamus: immunohistochemical localization. *Endocrinology* 1976; 98: 255-8.
 274. Merchenthaler I, Sétáló G, Csontos C, Petrusz P, Flerkó B, Negro-Vilar A. Combined retrograde tracing and immunocytochemical identification of luteinizing hormone-releasing hormone- and somatostatin-containing neurons projecting to the median eminence of the rat. *Endocrinology* 1989; 125: 2812-21.
 275. Patel YC, Greenwood MT, Panetta R, Demchyshyn L, Niznik H, Srikant CB. The somatostatin receptor family. *Life Sci* 1995; 57: 1249-65.
 276. Lussier BT, Wood DA, French MB, Moor BC, Kraicer J. Free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and growth hormone release from purified rat somatotrophs.II. Somatostatin lowers $[Ca^{2+}]_i$ by inhibiting Ca^{2+} influx. *Endocrinology* 1973; 128: 583-91.
 277. Sugihara H, Minami S, Okada K, Kamegai J, Hasegawa O, Wakabayashi I. Somatostatin reduces transcription of the growth hormone gene in rats. *Endocrinology* 1993; 132: 1225-9.
 278. Morishita M, Iwasaki Y, Onishi A, et al. The effects of GH-releasing hormone/somatostatin on the 5'-promoter

- activity of the GH gene in vitro. *J Mol Endocrinol* 2003; 31: 441-8.
279. Antoni FA. Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. *Endocr Rev* 1986; 7: 351-78.
280. Plotsky PM. Pathways to the secretion of adrenocorticotropin: a view from the portal. *J Neuroendocrinol* 1991; 3: 1-9.
281. Dallman MF. Stress update. Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends Endocrinol Metab* 1993; 4: 62-9.
282. Engler D, Redei E, Kola I. The corticotropin-release inhibitory factor hypothesis: a review of the evidence for the existence of inhibitory as well as stimulatory hypophysiotropic regulation of adrenocorticotropin secretion and biosynthesis. *Endocr Rev* 1999; 20: 460-500.
283. Volpi S, Rabadan-Diehl C, Aguilera G. Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis and stress adaptation. *Stress* 2004; 7: 75-83.
284. Jacobson L. Hypothalamic-pituitary-adrenocortical axis regulation. *Endocrinol Metab Clin North Am* 2005; 34: 327-39.
285. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and β -endorphin. *Science* 1981; 213: 1394-7.
286. Gillies GE, Linton EA, Lowry PJ. Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. *Nature* 1982; 299: 355-7.
287. Familiari M, Smith AI, Smith R, Funder JW. Arginine vasopressin is a much more potent stimulus to ACTH release from ovine anterior pituitary cells than ovine corticotropin-releasing factor. I. In vitro studies. *Neuroendocrinology* 1989; 50: 152-7.
288. Liu J-P, Robinson PJ, Funder JW, Engler D. The biosynthesis of adrenocorticotropin by the ovine anterior pituitary is predominantly regulated by arginine vasopressin (AVP). Evidence that protein kinase C mediates the action of AVP. *J Biol Chem* 1990; 265: 14136-42.
289. Kempainen RJ, Clark TP, Sartin JL, Zerbe CA. Hypothalamic peptide regulation of ACTH secretion from sheep pituitary. *Amer J Physiol* 1993; 265: R840-R845.
290. Bruhn TO, Sutton RE, Rivier CL, Vale WW. Corticotropin-releasing factor regulates proopiomelanocortin messenger ribonucleic acid levels in vivo. *Neuroendocrinology* 1984; 39: 170-5.
291. Höllt V, Haarmann I. Corticotropin-releasing factor differentially regulates pro-opiomelanocortin messenger ribonucleic acid levels in anterior as compared to intermediate pituitary lobes of rats. *Biochem Biophys Res Commun* 1984; 124: 407-15.
292. Gagner J-P, Drouin J. Opposite regulation of pro-opiomelanocortin gene transcription by glucocorticoids and CRH. *Mol Cell Endocrinol* 1985; 40: 25-32.
293. Boutillier AL, Monnier D, Lorang D, Lundblad JR, Roberts JL, Loeffler JP. Corticotropin-releasing hormone stimulates proopiomelanocortin transcription by cFos-dependent and -independent pathways: characterization of an AP-1 site in exon 1. *Mol Endocrinol* 1995; 9: 745-55.
294. Martens C, Bilodeau S, Maira M, Gauthier Y, Drouin J. Protein-protein interactions and transcriptional antagonism between the subfamily of BGFI-B/Nur77 orphan nuclear receptors and glucocorticoid receptor. *Mol Endocrinol* 2005; 19: 885-97.
295. Labrie F, Veilleux R, Lefevre R, Coy DH, Sueiras-Diaz J, Schally AV. Corticotropin-releasing factor stimulates accumulation of adenosine 3',5'-monophosphate in rat pituitary corticotrophs. *Science* 1982; 216: 1007-8.
296. Aguilera G, Harwood JP, Wilson JX, Morell J, Brown JH, Catt KJ. Mechanisms of action of corticotropin-releasing factor and other regulators of corticotropin release in rat pituitary cells. *J Biol Chem* 1983; 258: 8039-45.
297. Litvin Y, PasMantier R, Fleischer N, Erlichman J. Hormonal activation of the cAMP-dependent protein kinases in AtT20 cells. Preferential activation of protein kinase I by corticotropin releasing factor, isoproterenol, and forskolin. *J Biol Chem* 1984; 259: 10296-302.
298. Childs GV, Marchetti C, Brown AM. Involvement of sodium channels and two types of calcium channels in the regulation of adrenocorticotropin release. *Endocrinology* 1987; 120: 2059-69.
299. Marchetti C, Childs GV, Brown AM. Membrane currents of identified isolated rat corticotropes and gonadotropes. *Am J Physiol* 1987; 252: E340-E346.
300. Abou-Samra AB, Catt KJ, Aguilera G. Calcium-dependent control of corticotropin release in rat anterior pituitary cell cultures. *Endocrinology* 1987; 121:965-971.
301. Won JGS, Orth DN. Roles of intracellular and extracellular calcium in the kinetic profile of adrenocorticotropin secretion by perfused rat anterior pituitary cells. I. Corticotropin-releasing factor stimulation. *Endocrinology* 1990; 126: 849-57.
302. Guérineau N, Corcuff J-B, Tabarin A, Mollard P. Spontaneous and corticotropin-releasing factor-induced cytosolic calcium transients in corticotropes. *Endocrinology* 1991; 129: 409-20.
303. Antoni FA. Novel ligand specificity of pituitary vasopressin receptors in the rat. *Neuroendocrinology* 1981; 39: 186-8.
304. Gaillard RC, Schoenberg P, Favrod-Coune CA, et al. Properties of rat anterior pituitary vasopressin receptors: relation to adenylate cyclase and the effect of corticotropin-releasing factor. *Proc Natl Acad Sci USA* 1984; 81: 2907-11.
305. Koch B, Lutz-Bucher B. Specific receptors for vasopressin in the pituitary gland: evidence for down-regulation and desensitization to adrenocorticotropin-releasing factors. *Endocrinology* 1985; 116: 671-6.
306. Shen PJ, Clarke IJ, Canny BJ, Funder JW, Smith AI. Arginine vasopressin and corticotropin releasing factor: binding to ovine anterior pituitary membranes. *Endocrinology* 1990; 127: 2085-9.

307. Aguilera G, Pham Q, Rabadan-Diehl C. Regulation of pituitary vasopressin receptors during chronic stress: relationship with corticotroph responsiveness. *J Neuroendocrinol* 1994; 6: 299-304.
308. Sugimoto T, Saito M, Mochizuki S, Watanabe Y, Hashimoto S, Kawashima H. Molecular cloning and functional expression of a cDNA encoding the human V1b vasopressin receptor. *J Biol Chem* 1994; 269: 27088-92.
309. Berridge MJ, Irvine RF. Inositol phosphates and cell signalling. *Nature* 1989; 341: 197-205.
310. Nishizuka Y. Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 1992; 258: 607-14.
311. Liu J-P. Protein kinase C and its substrates. *Mol Cell Endocrinol* 1996; 116: 1-29.
312. Raymond V, Leung PCK, Veilleux R, Labrie F. Vasopressin rapidly stimulates phosphatidic acid-phosphatidylinositol turnover in rat anterior pituitary cells. *FEBS Lett* 1985; 182: 196-200.
313. Todd K, Lightman SL. Vasopressin activation of phosphatidylinositol metabolism in rat anterior pituitary in vitro and its modification by changes in the hypothalamo-pituitary-adrenal axis. *Neuroendocrinology* 1987; 45: 212-8.
314. Bilezikjian LM, Blount AL, Vale WW. The cellular actions of vasopressin on corticotrophs of the anterior pituitary: resistance to glucocorticoid action. *Mol Endocrinol* 1987; 1: 451-8.
315. Bilezikjian LM, Woodgett JR, Hunter T, Vale WW. Phorbol ester-induced down-regulation of protein kinase C abolishes vasopressin-mediated responses in rat anterior pituitary cells. *Mol Endocrinol* 1987; 1: 555-60.
316. Carvallo P, Aguilera G. Protein kinase C mediates the effect of vasopressin in pituitary corticotrophs. *Mol Endocrinol* 1989; 3: 1935-43.
317. Oki Y, Nicholson WE, Orth DN. Role of protein kinase C in the adrenocorticotropin secretory response to arginine vasopressin (AVP) and the synergistic response to AVP and corticotropin releasing factor by perfused rat anterior pituitary cells. *Endocrinology* 1990; 127: 350-7.
318. Liu J-P, Engler D, Funder JW, Robinson PJ. Evidence that the stimulation by arginine vasopressin (AVP) of the release of adrenocorticotropin (ACTH) from the ovine anterior pituitary involves the activation of protein kinase C. *Mol Cell Endocrinol* 1992; 87: 35-47.
319. Liu J-P, Engler D, Funder JW, Robinson PJ. Arginine vasopressin (AVP) causes the reversible phosphorylation of the Myristoylated Alanine Rich C Kinase Substrate (MARCKS) protein in the ovine anterior pituitary. Evidence that MARCKS phosphorylation is associated with adrenocorticotropin (ACTH) secretion. *Mol Cell Endocrinol* 1994; 105: 217-26.
320. Ben-Jonathan N, Oliver C, Weiner HJ, Mical RS, Porter JC. Dopamine in hypophysial portal plasma of the rat during the estrous cycle and throughout pregnancy. *Endocrinology* 1977; 100: 452-8.
321. Gibbs DM, Neill JD. Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. *Endocrinology* 1978; 102: 1895-900.
322. de Greef WJ, Klootwijk W, Karels B, Visser TJ. Levels of dopamine and thyrotrophin-releasing hormone in hypophysial stalk blood during an oestrogen-stimulated surge of prolactin in the ovariectomized rat. *J Endocrinol* 1985; 105: 107-12.
323. Ben-Jonathan N. Dopamine: a prolactin-inhibiting hormone. *Endocr Rev* 1985; 6: 564-89.
324. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. *Physiol Rev* 2000; 80: 1523-31.
325. Foord SM, Peters JR, Dieguez C, Scanlon MF, Hall R. Dopamine receptors on intact anterior pituitary cells in culture: functional association with inhibition of prolactin and thyrotropin. *Endocrinology* 1983; 112: 1567-77.
326. Malgaroli A, Vallar I, Elahi FR, Pozzan T, Spada A, Meldolesi J. Dopamine inhibits cytosolic Ca²⁺ increases in rat lactotroph cells. Evidence of a dual mechanism of action. *J Biol Chem* 1987; 262: 13920-7.
327. Jarvis WD, Judd AM, MacLeod RM. Attenuation of anterior pituitary phosphoinositide phosphorylase activity by the D₂ dopamine receptor. *Endocrinology* 1988; 123: 2793-9.
328. Lledo PM, Legendre P, Israel JM, Vincent JD. Dopamine inhibits two characterized voltage-dependent calcium currents in identified lactotroph cells. *Endocrinology* 1990; 127: 990-1001.
329. Haisenleder DJ, Moy JA, Gala RR, Lawson DM. The effect of transient dopamine antagonism on thyrotropin-releasing hormone-induced prolactin release in ovariectomized rats treated with estradiol and/or progesterone. *Endocrinology* 1986; 1996-2003.
330. Martinez de la Escalera G, Weiner RI. Dissociation of dopamine from its receptor as a signal in the pleiotropic hypothalamic regulation of prolactin secretion. *Endocr Rev* 1992; 13: 241-55.
331. Gudelsky GA, Porter JC. Release of dopamine from tuberoinfundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. *Endocrinology* 1980; 106: 526-9.
332. Demarest KT, Riegler GD, Moore KE. Hypoprolactinemia induced by hypophysectomy and long-term bromocriptine treatment decreases tuberoinfundibular dopaminergic neuronal activity and the responsiveness of these neurons to prolactin. *Neuroendocrinology* 1985; 40: 369-76.
333. Schally AV, Bowers CY, Redding TW, Barrett JF. Isolation of thyrotropin releasing factor (TRF) from porcine hypothalamus. *Biochem Biophys Res Commun* 1966; 25: 165-9.
334. Tashjian AH Jr, Barowsky NJ, Jensen DK. Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. *Biochem Biophys Res Commun* 1971; 43: 516-23.
335. Hinkle PM, Tashjian AH Jr. Receptors for thyrotropin-releasing hormone in prolactin-producing rat pituitary cells in culture. *J Biol Chem* 1973; 248: 6180-6.
336. Bowers CY, Friesen HG, Hwang P, Guyda HJ, Folkers K. Prolactin and thyrotropin release in man by synthetic py-

- roglutamyl-histidyl-prolinamide. *Biochem Biophys Res Commun* 1971; 45: 1033-41.
337. Oliver C, Ben-Jonathan N, Mical RS, Porter JC. Transport of thyrotropin-releasing hormone from cerebrospinal fluid to hypophysial portal blood and the release of thyrotropin. *Endocrinology* 1975; 97: 1138-43.
338. Harris AR, Christianson D, Smith MS, Fang SL, Braverman LE, Vagenakis AG. The physiological role of thyrotropin-releasing hormone in the regulation of thyroid-stimulating hormone and prolactin secretion in the rat. *J Clin Invest* 1978; 61: 441-8.
339. Fink G, Koch Y, Ben Aroya N. Release of thyrotropin-releasing hormone into hypophysial portal blood is high relative to other neuropeptides and may be related to prolactin secretion. *Brain Res* 1982; 243: 186-9.
340. Yu R, Ashworth R, Hinckle PM. Receptors for thyrotropin-releasing hormone on rat lactotropes and thyrotropes. *Thyroid* 1998; 8: 887-94.
341. Hsieh KP, Martin TF. Thyrotropin-releasing hormone and gonadotropin-releasing hormone receptors activate phospholipase C by coupling to the guanosine triphosphate-binding proteins G_q and G_{11} . *Mol Endocrinol* 1992; 6: 1673-81.
342. Fomina AF, Levitan ES. Three phases of TRH-induced facilitation of exocytosis by single lactotrophs. *J Neurosci* 1995; 15: 4982-91.
343. Said SI, Mutt V. Polypeptide with broad biological activity: isolation from small intestine. *Science* 1970; 169: 1217-8.
344. Larsson L-I, Fahrenkrug J, Schaffalitzky de Muckadell O, Sundler F, Håkanson R, Rehfeld JF. Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurons. *Proc Natl Acad Sci USA* 1976; 73: 3197-3200.
345. Mezey E, Kiss JZ. Vasoactive intestinal peptide-containing neurons in the paraventricular nucleus may participate in regulating prolactin secretion. *Proc Natl Acad Sci USA* 1985; 82: 245-7.
346. Said SI, Porter JC. Vasoactive intestinal polypeptide: release into hypophysial portal blood. *Life Sci* 1979; 24: 227-30.
347. Bjørø T, Wiik P, Opstad PK, Gautvik KM, Haug E. Binding and degradation of vasoactive intestinal peptide in prolactin-producing cultured rat pituitary tumour cells (GH_4C_1). *Acta Physiol Scand* 1987; 130: 609-18.
348. Gourdji D, Bataille D, Vauclin N, Grouselle D, Rosselin G, Tixier-Vidal A. Vasoactive intestinal peptide (VIP) stimulates prolactin (PRL) release and cAMP production in a rat pituitary cell line (GH_4B_6). Additive effects of VIP and TRH on PRL release. *FEBS Lett* 1979; 104: 165-8.
349. Aizawa T, Hinckle PM. Differential effects of thyrotropin-releasing hormone, vasoactive intestinal peptide, phorbol ester and depolarization in GH_4C_1 rat pituitary cells. *Endocrinology* 1985; 116: 909-19.
350. Prysor-Jones RA, Silverlight JJ, Jenkins JS. Vasoactive intestinal peptide increases intracellular free calcium in rat and human pituitary tumour cells *in vitro*. *J Endocrinol* 1987; 114: 119-23.
351. Sobel A, Tashjian AH Jr. Distinct patterns of cytoplasmic protein phosphorylation related to regulation of synthesis and release of prolactin by GH cells. *J Biol Chem* 1983; 258: 10312-24.
352. Abe H, Engler D, Molitch ME, Bollinger-Gruber J, Reichlin S. Vasoactive intestinal peptide is a physiological mediator of prolactin release in the rat. *Endocrinology* 1985; 116: 1383-90.
353. Zimmerman EA, Nilaver G, Hou-Yu A, Silverman A. Vasopressinergic and oxytocinergic pathways in the central nervous system. *Fed Proc* 1984; 43: 91-6.
354. Gibbs DM. High concentrations of oxytocin in hypophysial portal plasma. *Endocrinology* 1984; 114: 1216-8.
355. Horn AM, Robinson IC, Fink G. Oxytocin and vasopressin in rat hypophysial portal blood: experimental studies in normal and Brattleboro rats. *J Endocrinol* 1985; 104: 211-4.
356. Breton C, Pechoux C, Morel G, Zingg HH. Oxytocin receptor messenger ribonucleic acid: characterization, regulation, and cellular localization in the rat pituitary gland. *Endocrinology* 1995; 136: 2928-36.
357. Egli M, Bertram R, Sellix MT, Freeman ME. Rhythmic secretion of prolactin in rats: action of oxytocin coordinated by vasoactive intestinal polypeptide of suprachiasmatic nucleus origin. *Endocrinology* 2004; 145: 3386-94.
358. Samson WK, Lumpkin MD, McCann SM. Evidence for a physiological role for oxytocin in the control of prolactin secretion. *Endocrinology* 1986; 119: 554-60.
359. Johnston CA, Negro-Vilar A. Role of oxytocin on prolactin secretion during proestrus and in different physiological or pharmacological paradigms. *Endocrinology* 1988; 122: 341-50.
360. Swanson LW, Kuypers HGJM. The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. *J Comp Neurol* 1980; 194: 555-70.
361. van den Pol AN. The magnocellular and parvocellular paraventricular nucleus of rat: intrinsic organization. *J Comp Neurol* 1982; 206: 317-45.
362. Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci* 1983; 6: 269-324.
363. Sawchenko PE, Swanson LW. The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. *J Comp Neurol* 1983; 218: 121-44.
364. Cunningham ET Jr., Sawchenko PE. Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J Comp Neurol* 1988; 274: 60-76.
365. Cunningham ET Jr., Bohn MC, Sawchenko PE. Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Comp Neurol* 1990; 292: 651-67.

366. Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y - a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 1982; 296: 659-60.
367. Allen YS, Adrian TE, Allen JM, et al. Neuropeptide Y distribution in rat brain. *Science* 1983; 221: 877-9.
368. Chronwall BM, DiMaggio DA, Massari VJ, et al. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 1985; 15(4): 1159-81.
369. Sawchenko PE, Swanson LW, Grzanna R, Howe PRC, Bloom SR, Polak JM. Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. *J Comp Neurol* 1985; 242: 138-53.
370. Bai FL, Yamano M, Shiotani Y, et al. An arcuate-paraventricular and -dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. *Brain Res* 1985; 331: 172-5.
371. Jhanwar-Uniyal M, Beck B, Jhanwar YS, Burlet C, Leibowitz SF. Neuropeptide Y projection from arcuate nucleus to parvocellular division of paraventricular nucleus: specific relation to the ingestion of carbohydrate. *Brain Res* 1993; 631: 97-106.
372. Baker R, Herkenham M. Arcuate nucleus neurons that project to the hypothalamic paraventricular nucleus: neuropeptidergic identity and consequences of adrenalectomy on mRNA levels in the rat. *J Comp Neurol* 1995; 358: 518-30.
373. Tatemoto K, Rökæus A, Jörnvall H, McDonald TJ, Mutt V: Galanin-a novel biologically active peptide from porcine intestine. *FEBS Lett* 1983; 164: 124-8.
374. Skofitsch G, Jacobowitz DM. Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. *Peptides* 1985; 6: 509-46.
375. Melander T, Hökfelt T, Rökæus A, et al. Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. *J Neurosci* 1986; 6: 3640-54.
376. Melander T, Hökfelt T, Rökæus A. Distribution of galanin-like immunoreactivity in the rat central nervous system. *J Comp Neurol* 1986; 248: 473-517.
377. Levin MC, Sawchenko PE, Howe PR, Bloom SR, Polak JM. Organization of galanin-immunoreactive inputs to the paraventricular nucleus with special reference to their relationship to catecholaminergic afferents. *J Comp Neurol* 1987; 261: 562-82.
378. Kordower JH, Le HK, Mufson EJ. Galanin immunoreactivity in the primate central nervous system. *J Comp Neurol* 1992; 319: 479-500.
379. Perez SE, Wynick D, Steiner RA, Mufson EJ. Distribution of galaninergic immunoreactivity in the brain of the mouse. *J Comp Neurol* 2001; 28: 158-85.
380. Olschowka JA, O'Donohue TL, Mueller GP, Jacobowitz DM. The distribution of corticotropin releasing factor-like immunoreactive neurons in the rat brain. *Peptides* 1982; 3: 995-1015.
381. Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology* 1983; 36: 165-86.
382. Whitnall MH, Mezey E, Gainer H. Co-localization of corticotropin-releasing factor and vasopressin in median eminence secretory vesicles. *Nature* 1985; 317: 248-50.
383. Whitnall MH. Distribution of pro-vasopressin expressing and pro-vasopressin deficient CRH neurons in the paraventricular hypothalamic nucleus of colchicine-treated normal and adrenalectomized rats. *J Comp Neurol* 1988; 275: 13-28.
384. Liposits Z, Sievers L, Paull WK. Neuropeptide-Y and ACTH-immunoreactive innervation of corticotropin releasing factor (CRF)-synthesizing neurons in the hypothalamus of the rat. An immunocytochemical analysis at the light and electron microscopic levels. *Histochemistry* 1988; 88: 227-34.
385. Wittmann G, Liposits Z, Lechan RM, Fekete C. Origin of cocaine- and amphetamine-regulated transcript-containing axons innervating hypophysiotropic corticotropin-releasing hormone-synthesizing neurons in the rat. *Endocrinology* 2005; 146: 2985-91.
386. Wittmann G, Lechan RM, Liposits Z, Fekete C. Glutamatergic innervation of corticotropin-releasing hormone- and thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. *Brain Res* 2005; 1039: 53-62.
387. Sarkar S, Fekete C, Légrádi G, Lechan RM. Glucagon-like peptide-1 (7-36) amide (GLP-1) nerve terminals densely innervate corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *Brain Res* 2003; 985: 163-8.
388. Légrádi G, Hannibal J, Lechan RM. Pituitary adenylate cyclase-activating polypeptide-nerve terminals densely innervate corticotropin-releasing hormone-neurons in the hypothalamic paraventricular nucleus of the rat. *Neurosci Lett* 1998; 246: 145-8.
389. Lorén I, Emson PC, Fahrenkrug J, et al. Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. *Neuroscience* 1979; 4 (12): 1953-76.
390. Sims KB, Hoffman DL, Said SI, Zimmerman EA. Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: an immunocytochemical study. *Brain Res* 1980; 186: 165-83.
391. Obata-Tsuto HL, Okamura H, Tsuto T, et al. Distribution of the VIP-like immunoreactive neurons in the cat central nervous system. *Brain Res Bull* 1983; 10 (5): 653-60.
392. Okamura H, Murakami S, Fukui K, et al. Vasoactive intestinal peptide- and peptide histidine isoleucine amide-like immunoreactivity colocalize with vasopressin-like immunoreactivity in the canine hypothalamo-neurohypophysial neuronal system. *Neurosci Lett* 1986; 69: 227-32.
393. Ceccatelli S, Eriksson M, Hökfelt T. Distribution and coexistence of corticotropin-releasing factor-, neurotensin-, enkephalin-, cholecystokinin-, galanin- and vasoactive intestinal polypeptide/peptide histidine isoleucine-like peptides in the parvocellular part of the paraventricular nucleus. *Neuroendocrinology* 1989; 49: 309-23.

394. Ceccatelli S, Fahrenkrug J, Villar MJ, Hökfelt T. Vasoactive intestinal polypeptide/peptide histidine isoleucine immunoreactive neuron systems in the basal hypothalamus of the rat with special reference to the portal vasculature: an immunohistochemical and *in situ* hybridization study. *Neuroscience* 1991; 45: 483-502.
395. Toni R, Kakucska I, Mosca S, Marrama P, Lechan RM. Hypothyroidism increases vasoactive intestinal polypeptide (VIP) immunoreactivity and gene expression in the rat hypothalamic paraventricular nucleus. *Endocrinology* 1992; 131: 976-8.
396. Romijn HJ, van Eum JF, Emmering J, Goncharuk V, Buijs RM. Colocalization of VIP and AVP neurons of the human paraventricular, supraoptic and suprachiasmatic nucleus. *Brain Res* 1999; 832: 47-53.
397. Lechan RM, Jackson IMD. Immunohistochemical localization of thyrotropin-releasing hormone in the rat hypothalamus and pituitary. *Endocrinology* 1982; 111: 55-65.
398. Jackson IMD, Wu P, Lechan RM. Immunohistochemical localization in the rat brain of the precursor for thyrotropin-releasing hormone. *Science* 1985; 229: 1097-9.
399. Mihály E, Fekete C, Légrádi G, Lechan RM. Hypothalamic dorsomedial nucleus neurons innervate thyrotropin-releasing hormone-synthesizing neurons in the paraventricular nucleus. *Brain Res* 2001; 891: 20-31.
400. Kawano K, Tsuruo Y, Bando H, Daikoku S. Hypophysiotropic TRH-producing neurons identified by combining immunohistochemistry for pro-TRH and retrograde tracing. *J Comp Neurol* 1991; 307: 531-8.
401. Légrádi G, Lechan RM. The arcuate nucleus is the major source for neuropeptide Y-innervation of thyrotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *Endocrinology* 1998; 139: 3262-70.
402. Mihály E, Fekete C, Tatro JB, Liposits Z, Stopa EG, Lechan RM. Hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in the human hypothalamus are innervated by neuropeptide Y, agouti-related protein, and α -melanocyte-stimulating hormone. *J Clin Endocrinol Metab* 2000; 85: 2596-603.
403. Wittmann G, Liposits Z, Lechan RM, Fekete C. Medullary adrenergic neurons contribute to the neuropeptide Y-ergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in the rat. *Neurosci Lett* 2002; 324: 69-73.
404. Wittmann G, Sarkar S, Hrabovszky E, Liposits Z, Lechan RM, Fekete C. Galanin- but not galanin-like peptide-containing axon terminals innervate hypophysiotropic TRH-synthesizing neurons in the hypothalamic paraventricular nucleus. *Brain Res* 2004; 1002: 43-50.
405. Fekete C, Sarkar S, Lechan RM. Relative contribution of brainstem afferents to the cocaine- and amphetamine-regulated transcript (CART) innervation of thyrotropin-releasing hormone synthesizing neurons in the hypothalamic paraventricular nucleus (PVN). *Brain Res* 2005; 1032: 171-5.
406. Chronwall BM. Anatomy and physiology of the neuroendocrine arcuate nucleus. *Peptides* 1985; 6 Suppl 2: 1-11.
407. Everitt BJ, Meister B, Hökfelt T, et al. The hypothalamic arcuate nucleus-median eminence complex: immunohistochemistry of transmitters, peptides and DARPP-32 with special reference to coexistence in dopamine neurons. *Brain Res Rev* 1986; 11: 97-155.
408. Hisano S, Kagotani Y, Tsuruo Y, Daikoku S, Chihara K, Whitnall MH. Localization of glucocorticoid receptor in neuropeptide Y-containing neurons in the arcuate nucleus of the rat hypothalamus. *Neurosci Lett* 1988; 95: 13-8.
409. Sar M, Sahu A, Crowley WR, Kalra SP. Localization of neuropeptide-Y immunoreactivity in estradiol concentrating cells in the hypothalamus. *Endocrinology* 1990; 127: 2752-6.
410. Marks JL, Porte D Jr, Stahl WL, Baskin DG. Localization of insulin receptor mRNA in rat brain by *in situ* hybridization. *Endocrinology* 1990; 127: 3234-6.
411. Mercer JG, Hoggard N, Williams LM, et al. Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. *J Neuroendocrinol* 1996; 8: 733-5.
412. Häkansson M-L, Brown H, Ghilardi N, Skoda RC, Meister B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. *J Neurosci* 1998; 18: 559-72.
413. Kamegai J, Minami S, Sugihara H, Hasegawa O, Higuchi H, Wakabayashi I. Growth hormone receptor gene is expressed in neuropeptide Y neurons in hypothalamic arcuate nucleus of rats. *Endocrinology* 1996; 137: 2109-12.
414. Bloch B, Ling N, Benoit R, Wehrenberg WB, Guillemin R. Specific depletion of immunoreactive growth hormone-releasing factor by monosodium glutamate in rat median eminence. *Nature* 1984; 307: 272-3.
415. Smith RM, Howe PR, Oliver JR, Willoughby JO. Growth hormone releasing factor immunoreactivity in rat hypothalamus. *Neuropeptides* 1984; 4: 109-15.
416. Lechan RM, Lin HD, Ling N, Jackson IMD, Jacobson S, Reichlin S. Distribution of immunoreactive growth hormone releasing factor(1-44)NH₂ in the tuberoinfundibular system of the rhesus monkey. *Brain Res* 1984; 309: 55-61.
417. Sawchenko PE, Swanson LW, Rivier J, Vale WW. The distribution of growth-hormone-releasing factor (GRF) immunoreactivity in the central nervous system of the rat: an immunohistochemical study using antisera directed against rat hypothalamic GRF. *J Comp Neurol* 1985; 237: 100-15.
418. Seeley RJ, Drazan DL, Clegg DJ. The critical role of the melanocortin system in the control of energy balance. *Annu Rev Nutr* 2004; 24: 133-49.
419. Cone RD. Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 2005; 8(5): 571-8.
420. Iwai C, Ochiai H, Nakai Y. Electron-microscopic immunocytochemistry of neuropeptide Y immunoreactive innervation of vasopressin neurons in the paraventricular nucleus of the rat hypothalamus. *Acta Anat* 1989; 136 (4): 279-84.
421. Beroukas D, Willoughby JO, Blessing WW. Neuropeptide Y-like immunoreactivity is present in boutons syna-

- psing on vasopressin-containing neurons in rabbit supraoptic nucleus. *Neuroendocrinology* 1989; 50: 222-8.
422. Turi GF, Liposits Z, Moenter SM, Fekete C, Hrabovszky E. Origin of neuropeptide Y-containing afferents to gonadotropin-releasing hormone neurons in male mice. *Endocrinology* 2003; 144: 4967-74.
 423. Stanley BG, Kyrkouli SE, Lampert S, Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. *Peptides* 1986; 7: 1189-92.
 424. Sainsbury A, Schwarzer C, Couzens M, et al. Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knockout mice. *Proc Natl Acad Sci USA* 2002; 99: 8938-43.
 425. Egawa M, Yoshimatsu H, Bray GA. Neuropeptide Y suppresses sympathetic activity to interscapular brown adipose tissue in rats. *Am J Physiol* 1991; 260: R328-R334.
 426. Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev* 1997; 11: 593-602.
 427. Ollmann MM, Wilson BD, Yang Y-K, et al. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 1997; 278: 135-7.
 428. Lu D, Willard D, Patel IR, et al. Agouti protein is an antagonist of the melanocyte-stimulating hormone receptor. *Nature* 1994; 371: 799-802.
 429. Fong TM, Mao C, MacNeil T, et al. ART (protein product of agouti-related transcript) as an antagonist of MC-3 and MC-4 receptors. *Biochem Biophys Res Commun* 1997; 237: 629-31.
 430. Hahn TM, Breininger JF, Baskin DG, Schwartz MW. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1998; 1: 271-2.
 431. Broberger C, Johansen J, Johansson C, Schalling M, Hökfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci USA* 1998; 95: 15043-8.
 432. Haskell-Luevano C, Chen P, Li C, et al. Characterization of the neuroanatomical distribution of agouti-related protein immunoreactivity in the rhesus monkey and the rat. *Endocrinology* 1999; 140: 1408-15.
 433. Watson SJ, Akil H, Richard CW III, Barchas JD. Evidence for two separate opiate peptide neuronal systems and the coexistence of β -lipotropin, β -endorphin, ACTH immunoreactivities in the same hypothalamic neurons. *Nature* 1978; 275: 226-8.
 434. Jacobowitz DM, O'Donohue TL. α -melanocyte stimulating hormone: immunohistochemical identification and mapping in neurons of rat brain. *Proc Natl Acad Sci USA* 1978; 75: 6300-4.
 435. Nivaler G, Zimmerman EA, Defendini R, Liotta AS, Krieger DT, Brownstein MJ. Adrenocorticotropin and β -lipotropin in the hypothalamus. *J Cell Biol* 1979; 81: 50-8.
 436. Finley JCW, Lindstrom P, Petrusz P. Immunocytochemical localization of β -endorphin-containing neurons in the rat brain. *Neuroendocrinology* 1981; 33: 28-42.
 437. Liotta AS, Advis JP, Krause JE, McKelvy JF, Krieger DT. Demonstration of in vivo synthesis of pro-opiomelanocortin-, β -endorphin-, and α -melanotropin-like species in the adult rat brain. *J Neurosci* 1984; 4(4): 956-65.
 438. Raffin-Sanson ML, de Keyser Y, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. *Eur J Endocrinol* 2003; 149: 79-90.
 439. Hadley ME, Haskell-Luevano C. The proopiomelanocortin system. *Ann NY Acad Sci* 1999; 885: 1-21.
 440. Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol Endocrinol* 1994; 8: 1298-308.
 441. Kishi T, Aschkenasi CJ, Lee CE, Mountjoy KG, Saper CB, Elmquist JK. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. *J Comp Neurol* 2003; 457: 213-35.
 442. Douglass J, McKinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *J Neurosci* 1995; 15: 2471-81.
 443. Kristensen P, Judge ME, Thim L, et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 1998; 393: 72-6.
 444. Koylu EO, Couceyro PR, Lambert PD, Kuhar MJ. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. *J Comp Neurol* 1998; 391: 115-32.
 445. Elias CF, Lee CE, Kelly JF, et al. Characterization of CART neurons in the rat and human hypothalamus. *J Comp Neurol* 2001; 432: 1-19.
 446. Stanley SA, Small CJ, Murphy KG, et al. Actions of cocaine- and amphetamine-regulated transcript (CART) peptide on regulation of appetite and hypothalamo-pituitary axes in vitro and in vivo in male rats. *Brain Res* 2001; 893: 186-94.
 447. Raptis S, Fekete C, Sarkar S, et al. Cocaine- and amphetamine-regulated transcript co-contained in thyrotropin-releasing hormone (TRH) neurons of the hypothalamic paraventricular nucleus modulates TRH-induced prolactin secretion. *Endocrinology* 2004; 145: 1695-9.
 448. Smith SM, Vaughan JM, Donaldson CJ, et al. Cocaine- and amphetamine-regulated transcript activates the hypothalamic-pituitary-adrenal axis through a corticotropin-releasing factor receptor-dependent mechanism. *Endocrinology* 2004; 145: 5202-9.
 449. Fekete C, Sarkar S, Lechan RM. Relative contribution of brainstem afferents to the cocaine- and amphetamine-regulated transcript (CART) innervation of thyrotropin-releasing hormone synthesizing neurons in the hypothalamic paraventricular nucleus (PVN). *Brain Res* 2005; 1032: 171-5.
 450. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; 404: 661-71.

451. Benoit SC, Clegg DJ, Seeley RJ, Woods SC. Insulin and leptin as adiposity signals. *Recent Prog Horm Res* 2004; 59: 267-85.
452. Porte D Jr, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system. A critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes* 2005; 54: 1264-76.
453. Havrankova J, Roth J, Brownstein M. Insulin receptors are widely distributed in the central nervous system of the rat. *Nature* 1978; 272: 827-9.
454. Unger J, McNeill TH, Moxley RT III, et al. Distribution of insulin receptor-like immunoreactivity in the rat forebrain. *Neuroscience* 1989; 31 (1): 143-57.
455. Baura GD, Foster DM, Porte D Jr, et al. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. *J Clin Invest* 1993; 92: 1824-30.
456. Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* 1979; 282: 503-5.
457. Sipols AJ, Baskin DG, Schwartz MW. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* 1995; 44: 147-51.
458. Benoit SC, Air EL, Coolen LM, et al. The catabolic actions of insulin in the brain is mediated by melanocortins. *J Neurosci* 2002; 22: 9048-52.
459. Niswender KD, Morrison CD, Clegg DJ, et al. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus. A key mediator of insulin-induced anorexia. *Diabetes* 2003; 52: 227-31.
460. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 1995; 372: 425-32.
461. Licinio J, Mantzoros C, Negrão AB, et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nat Med* 1997; 3: 575-9.
462. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995; 269: 546-9.
463. Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the *obese* gene product on body weight regulation in *ob/ob* mice. *Science* 1995; 269: 540-3.
464. Tartaglia LA, Dembski M, Weng X, et al. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; 83: 1263-71.
465. Lee G-H, Proenca R, Montez JM, et al. Abnormal splicing of the leptin receptor in *diabetic* mice. *Nature* 1996; 379: 632-5.
466. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. *Peptides* 1996; 17: 305-11.
467. Golden PL, Maccagnan TJ, Pardridge WM. Human blood-brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. *J Clin Invest* 1997; 99: 14-8.
468. Bjørnbæk C, Elmquist JK, Michl P, et al. Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology* 1998; 139: 3485-91.
469. Kurrimbux D, Gaffen Z, Farrell CL, Martin D, Thomas SA. The involvement of the blood-brain and the blood-cerebrospinal fluid barriers in the distribution of leptin into and out of the rat brain. *Neuroscience* 2004; 123: 527-36.
470. Fei H, Okano HJ, Li C, et al. Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc Natl Acad Sci USA* 1997; 94: 7001-5.
471. Elmquist JK, Bjørnbæk C, Ahima RS, Flier JS, Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. *J Comp Neurol* 1998; 395: 535-47.
472. Cohen P, Zhao C, Cai X, et al. Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 2001; 108: 1113-21.
473. Bjørnbæk C, Kahn BB. Leptin signaling in the central nervous system and the periphery. *Recent Prog Horm Res* 2004; 59: 305-31.
474. Myers MG Jr. Leptin receptor signaling and the regulation of mammalian physiology. *Recent Prog Horm Res* 2004; 59: 287-304.
475. Banks AS, Davis SM, Bates SH, Myers MG Jr. Activation of downstream signals by the long form of the leptin receptor. *J Biol Chem* 2000; 275: 14563-72.
476. Dunn SL, Björnholm M, Bates SH, Chen Z, Seifert M, Myers MG Jr. Feedback inhibition of leptin receptor/Jak2 signaling via Tyr₁₁₃₈ of the leptin receptor and suppressor of cytokine signaling 3. *Mol Endocrinol* 2005; 19:925-38.
477. Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG Jr, Schwartz MW. Intracellular signaling: Key enzyme in leptin-induced anorexia. *Nature* 2001; 413: 794-5.
478. Zhao AZ, Huan JN, Gupta S, Pal R, Sahu A. A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding. *Nat Neurosci* 2002; 5: 727-8.
479. Wang Q, Bing C, Al-Barazanji K, et al. Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes* 1997; 46: 335-41.
480. Elias CF, Aschkenasi C, Lee C, et al. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* 1999; 23: 775-86.
481. Wilson BD, Bagnol D, Kaelin CB, et al. Physiological and anatomical circuitry between agouti-related protein and leptin signaling. *Endocrinology* 1999; 140: 2387-97.
482. Baskin DG, Breininger JF, Schwartz MW. Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. *Diabetes* 1999; 48: 828-33.
483. Elias CF, Kelly JF, Lee CE, et al. Chemical characterization of leptin-activated neurons in the rat brain. *J Comp Neurol* 2000; 423: 261-81.
484. Cowley MA, Smart JL, Rubinstein M, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 2001; 411: 480-4.

485. Xu AW, Kaelin CB, Takeda K, Akira S, Schwartz MW, Barsh GS. PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest* 2005; 115: 951-8.
486. Thompson RH, Swanson LW. Organization of inputs to the dorsomedial nucleus of the hypothalamus: a reexamination with Fluorogold and PHAL in the rat. *Brain Res Rev* 1998; 27: 89-118.
487. Thompson RH, Canteras NS, Swanson LW. Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHAL study in the rat. *J Comp Neurol* 1996; 376: 143-73.
488. Levitsky DA. Feeding patterns of rats in response to fasts and changes in environmental conditions. *Physiol Behav* 1970; 5: 291-300.
489. Mueller DT, Loft A, Eikelboom R. Alternate-day wheel access: effects on feeding, body weight, and running. *Physiol Behav* 1997; 62: 905-8.
490. Kawaguchi M, Scott KA, Moran TH, Bi S. Dorsomedial hypothalamic corticotropin-releasing factor mediation of exercise-induced anorexia. *Am J Physiol* 2005; 288: R1800-R1805.
491. Rivest S, Richard D. Involvement of corticotropin-releasing factor in the anorexia induced by exercise. *Brain Res Bull* 1990; 25: 169-72.
492. Rivest S, Richard D. Hypothalamic paraventricular nucleus lesions do not prevent anorectic effect of exercise in male rats. *Am J Physiol* 1990; 259: R579-R584.
493. Bovetto S, Richard D. Lesion of central nucleus of amygdala promotes fat gain without preventing effect of exercise on energy balance. *Am J Physiol* 1995; 269: R781-R786.
494. Lewis DE, Shellard L, Koeslag DG, et al. Intense exercise and food restriction cause similar hypothalamic neuro-peptide Y increases in rats. *Am J Physiol* 1993; 264: E279-E284.
495. Hickey MS, Considine RV, Israel RG, et al. Leptin is related to body fat content in male distance runners. *Am J Physiol* 1996; 271: E938-E940.
496. Perusse L, Collier G, Gagnon J, et al. Acute and chronic effects of exercise on leptin levels in humans. *J Appl Physiol* 1997; 83: 5-10.
497. Leal-Cerro A, Garcia-Luna PP, Astorga R, et al. Serum leptin levels in male marathon athletes before and after the marathon run. *J Clin Endocrinol Metab* 1998; 83: 2376-9.
498. Thong FS, Hudson R, Ross R, Janssen I, Graham TE. Plasma leptin in moderately obese men: Independent effects of weight loss and aerobic exercise. *Am J Physiol* 2000; E307-E313.
499. Hulver MW, Houmard JA. Plasma leptin and exercise: recent findings. *Sports Med* 2003; 33: 473-82.
500. O'Neal HA, Van Hoomissen JD, Holmes PV, Dishman RK. Prepro-galanin messenger RNA levels are increased in rat locus coeruleus after treadmill exercise training. *Neurosci Lett* 2001; 299: 69-72.
501. Merchenthaler I. Neurons with access to the general circulation in the central nervous system of the rat: a retrograde tracing study with fluorogold. *Neuroscience* 1991; 44: 655-62.
502. Pardridge WM. Receptor-mediated transport through the blood-brain barrier. *Endocr Rev* 1987; 7: 314-30.
503. King GL, Johnson SM. Receptor-mediated transport of insulin across endothelial cells. *Science* 1985; 277: 1583-6.
504. Schwartz MW, Bergman RN, Kahn SE, et al. Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. *J Clin Invest* 1991; 88: 1272-81.
505. Woods SC, Seeley RJ, Baskin DG, Schwartz MW. Insulin and the blood-brain barrier. *Curr Pharm Des* 2003; 9 (10): 795-800.
506. Murakami Y, Kato Y, Koshiyama H, Inoue T, Yanaihara N, Imura H. Galanin stimulates growth hormone (GH) secretion via GH-releasing factor (GRF) in conscious rats. *Eur J Pharmacol* 1987; 136: 415-8.
507. Davis TM, Burrin JM, Bloom SR. Growth hormone (GH) release in response to GH-releasing hormone in man is 3-fold enhanced by galanin. *J Clin Endocrinol Metab* 1987; 65: 1248-52.
508. Murakami Y, Kato Y, Shimatsu A, et al. Possible mechanisms involved in growth hormone secretion induced by galanin in the rat. *Endocrinology* 1989; 124: 1224-9.
509. Niimi M, Takahara J, Sato M, Kawanishi K. Immunohistochemical identification of galanin and growth hormone-releasing factor-containing neurons projecting to the median eminence of the rat. *Neuroendocrinology* 1990; 51: 572-5.
510. Maiter DM, Hooi SC, Koenig JJ, Martin JB. Galanin is a physiological regulator of spontaneous pulsatile secretion of growth hormone in the male rat. *Endocrinology* 1990; 126: 1216-22.
511. Tanoh T, Shimatsu A, Ishikawa Y, Ihara C, Yanaihara N, Imura H. Galanin-induced growth hormone secretion in conscious rats: evidence for a possible involvement of somatostatin. *J Neuroendocrinol* 1993; 5: 183-7.
512. Giustina A, Licini M, Schettino M, Doga M, Pizzoccolo G, Negro-Vilar A. Physiological role of galanin in the regulation of anterior pituitary function in humans. *Am J Physiol* 1994; 266: E57-E61.
513. Chan YY, Grafstein-Dunn E, Deleamarre-Van de Waal HA, Burton KA, Clifton DK, Steiner RA. The role of galanin and its receptor in the feedback regulation of growth hormone secretion. *Endocrinology* 1996; 137: 5303-10.
514. Liposits Z, Merchenthaler I, Reid JJ, Negro-Vilar A. Galanin-immunoreactive axons innervate somatostatin-synthesizing neurons in the anterior periventricular nucleus of the rat. *Endocrinology* 1993; 132: 917-23.
515. Liu J-P, Clarke IJ, Funder JW, Engler D. Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the hypophysial-portal circulation of the conscious sheep. II. The central noradrenergic and neuro-peptide Y pathways cause immediate and prolonged hypothalamic-pituitary-adrenal activation. Potential involvement in the pseudo-Cushing's syndrome of endogenous

- depression and anorexia nervosa. *J Clin Invest* 1994; 93: 1439-50.
516. Dunn AL, Reigle TG, Youngstedt SD, Armstrong RB, Dishman RK. Brain norepinephrine and metabolites after treadmill training and wheel running in rats. *Med Sci Sports Exerc* 1996; 28: 204-9.
517. Dishman RK, Renner KJ, White-Welkley JE, Burke KA, Bunnell BN. Treadmill exercise training augments brain norepinephrine response to familiar and novel stress. *Brain Res Bull* 2000; 52: 337-42.
518. Wahlestedt C, Skagerberg G, Ekman R, Heilig M, Sundler F, Håkanson R. Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. *Brain Res* 1987; 417: 33-8.
519. Inui A, Inoue T, Nakajima M, et al. Brain neuropeptide Y in the control of adrenocorticotrophic hormone secretion in the dog. *Brain Res* 1990; 510: 211-5.
520. Koshiyama H, Kato Y, Inoue T, et al. Central galanin stimulates pituitary prolactin secretion in rats: possible involvement of hypothalamic vasoactive intestinal polypeptide. *Neurosci Lett* 1987; 75: 49-54.
521. Inoue T, Kato Y, Koshiyama H, Yanaihara N, Imura H. Galanin stimulates the release of vasoactive intestinal polypeptide from perfused hypothalamic fragments in vitro and from periventricular structures into the cerebrospinal fluid in vivo in the rat. *Neurosci Lett* 1988; 85: 95-100.
522. Ottlecz A, Snyder GD, McCann SM. Regulatory role of galanin in control of hypothalamic-anterior pituitary function. *Proc Natl Acad Sci USA* 1988; 85: 9861-5.
523. van der Beek EM, Swarts HJ, Wiegant VM. Central administration of antiserum to vasoactive intestinal peptide delays and reduces luteinizing hormone and prolactin surges in ovariectomized, estrogen-treated rats. *Neuroendocrinology* 1999; 69: 227-37.
524. Meeusen R, De Meirleir K. Exercise and brain neurotransmission. *Sports Med* 1995; 20: 160-88.
525. Meeusen R, Smolders I, Sarre S, et al. Endurance training effects on neurotransmitter release in rat striatum: an in vivo microdialysis study. *Acta Physiol Scand* 1997; 159: 335-41.
526. Gopalan C, Tian Y, Moore KE, Lookingland KJ. Neurochemical evidence that the inhibitory effect of galanin on tuberoinfundibular dopamine neurons is activity dependent. *Neuroendocrinology* 1993; 58: 287-93.
527. Bi S, Scott KA, Hyun J, Ladenheim EE, Moran TH. Running wheel activity prevents hyperphagia and obesity in Otsuka Long-Evans Tokushima fatty rats: role of hypothalamic signaling. *Endocrinology* 2005; 146: 1676-85.
528. Elmquist JK, Ahima RS, Elias CF, Flier JS, Saper CB. Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci USA* 1998; 95: 741-6.
529. White JD, Olchovsky D, Kershaw M, Berelowitz M. Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. *Endocrinology* 1990; 126: 765-72.
530. Mezey E, Kiss JZ, Mueller GP, Eskay R, O'Donohue TL, Palkovits M. Distribution of the pro-opiomelanocortin derived peptides, adrenocorticotrope hormone, α -melanocyte-stimulating hormone and β -endorphin (ACTH, α -MSH, β -END) in the rat hypothalamus. *Brain Res* 1985; 328: 341-7.
531. Singru PS, Fekete C, Lechan RM. Neuroanatomical evidence for participation of the hypothalamic dorsomedial nucleus (DMN) in regulation of the hypothalamic paraventricular nucleus (PVN) by α -melanocyte stimulating hormone. *Brain Res* 2005; 1064: 42-51.
532. Champagne D, Beaulieu J, Drolet G. CRFergic innervation of the paraventricular nucleus of the rat hypothalamus: a tract-tracing study. *J Neuroendocrinol* 1998; 10: 119-31.
533. Sarkar S, Légrádi G, Lechan RM. Intracerebroventricular administration of α -melanocyte stimulating hormone increases phosphorylation of CREB in TRH- and CRH-producing neurons of the hypothalamic paraventricular nucleus. *Brain Res* 2002; 945: 50-9.
534. Lu X-Y, Barsh GS, Akil H, Watson SJ. Interaction between α -melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. *J Neurosci* 2003; 23: 7863-72.
535. Kishi T, Aschkenasi CJ, Lee CE, Mountjoy KG, Saper CB, Elmquist JK. Expression of melanocortin receptor mRNA in the central nervous system of the rat. *J Comp Neurol* 2003; 457: 213-35.
536. Chen P, Williams SM, Grove KL, Smith MS. Melanocortin 4 receptor-mediated hyperphagia and activation of neuropeptide Y expression in the dorsomedial hypothalamus during lactation. *J Neurosci* 2004; 24: 5091-100.
537. Kesterson RA, Huszar D, Lynch CA, Simerly RB, Cone RD. Induction of neuropeptide Y gene expression in the dorsal medial hypothalamic nucleus in two models of the *agouti* obesity syndrome. *Mol Endocrinol* 1997; 11: 630-7.
538. Guan XM, Yu H, Van der Ploeg LH. Evidence of altered hypothalamic pro-opiomelanocortin/neuropeptide Y mRNA expression in tubby mice. *Brain Res Mol Brain Res* 1998; 59: 273-9.
539. Tritos NA, Elmquist JK, Mastaitis JW, Flier JS, Maratos-Flier E. Characterization of expression of hypothalamic appetite-regulating peptides in obese hyperleptinemic brown adipose tissue-deficient (uncoupling protein-promoter-driven diphtheria toxin A) mice. *Endocrinology* 1998; 139: 4634-41.
540. Lambert PD, Wilding JP, al-Dokhayel AA, Gilbey SG, Ghatei MA, Bloom SR. Naloxone-induced anorexia increases neuropeptide Y concentrations in the dorsomedial hypothalamus: evidence for neuropeptide Y-opioid interactions in the control of food intake. *Peptides* 1994; 15: 657-60.
541. Guan XM, Yu H, Trumbauer M, Frazier E, Van der Ploeg LH, Chen H. Induction of neuropeptide Y expression in dorsomedial hypothalamus of diet-induced obese mice. *Neuroreport* 1998; 9: 3415-9.

542. Li C, Chen P, Smith MS. The acute suckling stimulus induces expression of neuropeptide Y (NPY) in cells in the dorsomedial hypothalamus and increases NPY expression in the arcuate nucleus. *Endocrinology* 1998; 139: 1645-52.
543. Chen P, Smith MS. Suckling-induced activation of neuronal input to the dorsomedial nucleus of the hypothalamus: possible candidates for mediating the activation of DMH neuropeptide Y neurons during lactation. *Brain Res* 2003; 984: 11-20.
544. Chen P, Smith MS. Regulation of hypothalamic neuropeptide Y messenger ribonucleic acid expression during lactation: role of prolactin. *Endocrinology* 2004; 145: 823-9.
545. Smith MS. Lactation alters neuropeptide-Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat. *Endocrinology* 1993; 133: 1258-65.
546. Morrison CD, Morton GJ, Niswender KD, Gelling RW, Schwartz MW. Leptin inhibits hypothalamic Npy and Agrp gene expression via a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. *Amer J Physiol* 2005; 289: E1051-E1057.
547. Bi S, Robinson BM, Moran TH. Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. *Amer J Physiol* 2003; 285: R1030-R1036.
548. Li C, Chen P, Smith MS. Neuropeptide Y and tuberoinfundibular dopamine activities are altered during lactation: role of prolactin. *Endocrinology* 1999; 140: 118-23.
549. Zheng D, Wootter MH, Zhou Q, Dohm GL. The effect of exercise on ob gene expression. *Biochem Biophys Res Commun* 1996; 225: 747-50.
550. Landt M, Lawson GM, Helgeson JM, et al. Prolonged exercise decreases serum leptin concentrations. *Metabolism* 1997; 46: 1109-12.
551. Nara M, Kanda T, Tsukui S, et al. Reduction of leptin precedes fat loss from running exercise in insulin resistant rats. *Exp Clin Endocrinol Diabetes* 1999; 107: 431-4.
552. Zimmet P, Alberti KGMM, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; 414: 782-7.
553. Cameron AJ, Wellborn TA, Zimmet PZ, et al. Overweight and obesity in Australia: the 1999-2000 Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Med J Aust* 2004; 178: 427-32.
554. Liu L, Ikeda K, Chen M, et al. Obesity, emerging risk in China: trend of increasing prevalence of obesity and its association with hypertension and hypercholesterolaemia among the Chinese. *Clin Exp Pharmacol Physiol* 2004; 31 Suppl 2: S8-S10.
555. Morrill AC, Chinn CD. The obesity epidemic in the United States. *J Public Health Policy* 2004; 25: 353-66.
556. Olshansky SJ, Passaro DJ, Hershow RC, et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* 2005; 352: 1138-45.
557. Morabia A, Costanza MC. The obesity epidemic as harbinger of a metabolic disorder epidemic: trends in overweight, hypercholesterolemia, and diabetes treatment in Geneva, Switzerland, 1993-2003. *Am J Public Health* 2005; 95: 632-5.
558. Smith AM, Lopez-Jimenez F, McMahon MM, et al. Action on obesity: Report of a Mayo Clinic National Summit. *Mayo Clin Proc* 2005; 80: 527-32.
559. Islam N. Obesity: an epidemic of the 21st century. *J Pak Med Assoc* 2005 55: 118-23.
560. Haslam DW, James WP. Obesity. *Lancet* 2005; 366: 1197-209.

Correspondence: Dennis Engler MD,
Private Consulting Suite B,
Monash Medical Centre,
246 Clayton Road,
Clayton, Victoria, Australia 3168
Tel. 61-3-9594 2461
Fax 61-3-9594 6475
E-mail: dennis.engler@med.monash.edu.au