

Central nervous system recognition of peripheral inflammation: a neural, hormonal collaboration

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Abstract. A key question in understanding communication between the immune system and the brain is: how does the central nervous system (CNS) recognise the extent of inflammatory or other immune responses taking place in peripheral tissues, so that it may respond appropriately? A variety of experimental models, particularly of fever, hypothalamus pituitary adrenal axis (HPAA) activation and sickness behaviour have been employed to investigate this. Although each of these responses is, at least in part, regulated in different areas of the CNS, they all require common information about peripheral inflammation in order to mount a coordinated response. Cytokines produced during the inflammatory response have been proposed as the important humoral mediators by which this is achieved. The experimental systems employed to investigate this have been dominated largely by examination of responses to bacterial lipopolysaccharide (LPS), which produces robust responses but does not make it easy to track down communication pathways when the LPS reaches the circulation. The data has been quite varied and several mediators and mechanisms have been considered responsible for interacting with the CNS, possibly because each plays a role at some level and their importance depends on the experimental system being examined. Both cytokines and LPS are able to induce the production of prostaglandins, which can in turn modify neural afferent pathways affecting CNS responses. Accompanying the variable dependence of these responses on neural regulation, the main endocrine input that arises from inflammatory tissues seems attributable to interleukin-6 (IL-6). This may gain direct access to the CNS but appears primarily to act by inducing the brain microvasculature to produce prostaglandin E₂, which then alters the activity of neurons controlling body temperature, HPAA and behavioural responses. (www.actabiomedica.it)

Key words: Cytokines, fever, hypothalamus pituitary adrenal axis, lipopolysaccharide, sickness behaviour

Introduction

Many research groups have turned their minds to the issue of how the central nervous system (CNS) responds to inflammation. In doing so, temperature regulation and the hypothalamus pituitary adrenal axis (HPAA) response have been explored most widely (1-4), but responses under the general heading of sickness behaviour (e.g. activity, anorexia, somnolence, hyperalgesia and allodynia) and immune regulation have also contributed importantly to the field (5-9). The mechanisms that link peripheral tissue inflammation to these CNS responses appear to have a number

of common features, but there is a surprising lack of agreement over the pathways and mechanisms involved. This review reflects primarily on experimental studies of fever, the HPAA and behaviour, to examine why this is the case and the extent to the data allow an explanation of how effective communication between inflammation and the CNS is achieved.

The experimental models

By far the most common inflammatory stimulus used for investigation of CNS responses has been bac-

terial lipopolysaccharide (LPS), usually injected intravenously (iv), or intraperitoneally (ip). The rationale is that it mimics infectious challenge, and sepsis when injected systemically. However, whereas inflammation usually starts as a localised tissue response to infection or trauma, injected LPS may course throughout the vasculature and act on organs such as the liver and brain within minutes of challenge. This immediately presents a problem when considering mechanisms by which inflammatory signals pass from tissues to the brain, because the Toll-like receptors (TLR) that respond to LPS and other pathogen-derived molecules are present in the circumventricular organs (CVOs), choroid plexus and leptomeninges of the brain (10). Some groups have placed stimuli more locally, either intramuscular (im) or into an air pouch, or have injected substances, such as turpentine (TPS) into tissues (11-13), but such experiments have been the minority.

The advantage of studying fever is that the responses are quite easy to monitor continuously, in a relatively non-invasive manner. Such experimental studies have primarily been conducted in rats, mice and guinea pigs, and the extreme variability in observed responses merits some comment. In rats responses to intravenous (iv) or intraperitoneal (ip) LPS are usually bi-phasic or tri-phasic (14-18), although the initial phase sometimes appears attributable to stress, since it is seen in animals injected with saline. In some cases, however, a single phase of fever is apparent (19). Mice are principally resorted to for fever experiments so that availability of gene deficient animals or species-specific reagents can be exploited, and the responses in these animals appear more complex. Small animals have to work harder to maintain body temperature and some groups find fevers in mice very difficult to reproduce (personal communications). Some perform their experiments in the thermoneutral zone, at about 30°C, and induce fevers while the mice are in their inactive phase of the circadian cycle (13, 20), while others appear to be able to induce fever at lower temperatures (21, 22). In both situations the fevers seem to have a single LPS-induced component, although the experiments performed at higher environmental temperatures often have a large immediate peak in temperature, apparently due to stress. In guinea pigs, the response to LPS is generally bi-phasic, with peaks

at approximately one and 3h after injection, whether LPS is iv, ip or im, although responses seem more rapid after the iv route (23, 24). Interestingly, injection of iv LPS into humans induces a uniphasic fever, peaking at about 3h (25, 26) and with a lag phase of about an hour. These comments do not take into account other experimental differences, most notably the different doses of LPS used, and are really here to make the point that there is variability between the experimental systems, even where the stimulus is so apparently similar. It is therefore not surprising there is some conflict over interpretation of data.

The nervous system

Before considering endocrine and other hormonal factors involved in promoting these responses, a brief acknowledgement of the role of neural afferent stimulation is appropriate. This is clearly a major route of activation for the HPA axis, which can be activated rapidly, without need to invoke any humoral signals from inflammatory foci. Neural afferent signals from peripheral nociceptors and from higher centres in the CNS rapidly integrate a response that triggers corticotrophin releasing hormone (CRH) and vasopressin release from the hypothalamus, following even the most minor stress and trauma (2). Although neural mechanisms of fever induction are less obvious, rapid stress-induced increases in body temperature have already been alluded to. These could hardly be ascribed to a systemic hormonal mechanism and there is evidence that at least some types of stress induce true fevers (27). Sickness behaviour also has an important neural component, a major stimulus being the pain that frequently accompanies inflammation and this may be one of the most important triggers (6, 28). However, neural signals have acquired additional significance in the context of sickness behaviour (29), as well as the other CNS responses, and will be described further below.

Peripheral cytokines

Circulating mediators of inflammatory and immune responses have been considered as potential

modifiers of CNS responses since the pioneering experiments of Besedovsky and colleagues in the 1970's (30-32). These achieved particular attention when it was demonstrated that interleukin (IL 1) could reproduce some of these actions and activate the HPA axis in particular (33, 34). Interestingly, cytokines are often considered not to be true hormones, although they clearly meet the necessary criteria (35). Certainly most cytokines do not seem to have an endocrine function, although some do. The lack of clarity over their endocrine role has been a particular source of confusion in considering the role of cytokines in inflammation, especially in terms of explaining effects on the CNS. This issue is clearly compounded if one cannot localise where the inflammatory stimulus that is inducing these hormones is itself acting.

Including some 50 chemokines, and 30 interleukins, the number of characterised cytokines is now well in excess of 100 (36). Many of these are particularly associated with regulation of the adaptive immune system and anti-viral responses, or act as growth and repair factors, and the great majority act primarily within the tissue where inflammation arises (37). However, despite their number, the focus in terms of the responses under discussion has remained quite firmly with IL-1, IL-6 and tumour necrosis factor α (TNF α) (2, 4, 6). [Note: for the purposes of this review use of the term TNF generally means TNF α , but is used where there is either uncertainty because an assay cannot distinguish it from other forms, or in re-

spect of the receptor, which binds other forms. The term IL-1 rather than IL-1 β , is used for similar reasons, or because the action described might be just as true for IL-1 α]. These cytokines exemplify the pleiotropy and redundancy that characterises many cytokines (Table 1), but it is important to note that ascribed properties are generally pharmacological and do not necessarily equate to a general physiological or pathophysiological role. This mismatch has sometimes led to sweeping statements about action that seem to defy the facts. Probably the most blatant of these, still seen in many reviews and textbooks, is that IL-1 circulates to the CNS and mediates a variety of effects. If this occurs at all it is very rare, and occurs primarily in experimental systems where either a high dose of LPS or IL-1 itself has been injected. Just because IL-1 is produced in most inflammatory situations, is a key mediator of inflammation, and can induce CNS activation, does not mean the IL-1 produced at the inflammatory site impacts directly upon the CNS. Therefore, and as will be discussed further below, the last four actions in the table generally do not occur in respect of IL-1 or TNF α , unless these cytokines are produced in the target tissue itself.

Whether triggered by infection or trauma, cells of the monocyte/macrophage lineage are generally the first to respond and produce these cytokines. They also produce the only natural and well characterised competitive cytokine antagonist, IL-1 receptor antagonist (IL-1ra) and liberate soluble forms of the TNF

Table 1. Exemplar Actions of 'Inflammatory' Cytokines

Locus of Action	Biological Action	TNF- α	IL-1	IL-6
Site of Tissue Inflammation	COX-2 and iNOS synthase induction	+	++	-
	Endothelial cell activation	+	++	+
	Proteolytic enzyme induction	+	++	-
	Cytokine induction	+	++	-
Lymphoid Tissues	Lymphocyte regulation	\pm	\pm	+
Systemic	Acute phase protein induction	+	++	++
	CRH/ACTH/ glucocorticoid	\pm	++	+
	Induction of fever	+	++	+
	Cachexia/anorexia	+	-	+
	Myelopoiesis	\pm	+	++

COX-2 = Cyclooxygenase 2; CRH = Corticotropin releasing hormone; iNOS = inducible nitric oxide; ACTH = Adrenocorticotrophic hormone. + and - symbols represent relative capacity to effect action

and IL-1 receptors that are able to bind and neutralise TNF α and IL-1 respectively. A soluble form of the IL-6 receptor is also produced (sIL-6R), although it does not neutralise IL-6 and binds it in a biologically active state (38). IL-1 and TNF α are able to induce further IL-6 production in surrounding connective tissue and endothelial cells, which explains why IL-6 is almost always found in far higher concentrations than TNF α or IL-1 during an inflammatory response. As suggested above, IL-1 and TNF α are almost never found in biologically significant amounts (39, 40) in plasma, unless the stimulus (such as LPS) is present systemically (26, 41). Since LPS and IL-1 activate similar intracellular signalling pathways, via receptors requiring related adapter proteins (42), it can be difficult to identify which is responsible for tissue activation (10). In contrast, high levels of synthesis, and protection by its soluble receptor (sIL-6R), partially explains why IL-6 is ideally suited to an endocrine function, its most well defined function in this respect being to act as the major inducer of acute phase proteins by hepatocytes (43). IL-1 and TNF α are also able to do this, but physiologically this only happens when the inflammatory stimulus, such as LPS, is able to reach the liver and activate Kupffer cells to produce these cytokines directly (44).

Central cytokines

Rather than macrophages, the innate immune system of the CNS is represented by microglial cells, although there are also macrophages around many of the blood vessels and these are certainly capable of producing cytokines such as IL-1 and IL-6 (45-47). Production by these cells is one of the few ways that these cytokines can enter the CNS, since the blood brain barrier (BBB) excludes entry of such proteins. An exception is in regions where the BBB is not well formed, such as around the circumventricular organs (CVOs), the meninges and the choroid plexus. Transport of cytokines across the BBB has been described (48, 49), but this is at such a low level it cannot be considered a realistic means of entry, unless plasma cytokine concentrations are maintained at a high concentration, and for most cytokines this is not the case.

Interpretation is again complicated where substances such as LPS reach the brain microvasculature, but essentially the only locus of action for blood cytokines, should they reach the brain, is the brain vasculature and the CVOs.

IL-1 and the CNS

Nevertheless, IL-1 has been demonstrated in the brain, following injection of LPS. Van Dam et al. detected IL-1 β in brain after iv injection of 0.01-2.5 mg/kg LPS (The exact effective doses are not clear, but the lowest was not effective (47)). The IL-1 β immunostaining was observed mostly in blood vessel of the meninges and choroids plexus, with some in non-neuronal cells of parenchyma. Little was seen in the hypothalamus and, where detected, was seen only after 4h. A few cells observed in parenchyma and in subsequent experiments were observed to be intermediate between macrophage and microglial morphology (50). Quan et al. also only detected IL-1 activity in brain at 6h (51). Other studies have found IL-1 β in homogenised hypothalamus at 3 to 4h after administration of LPS (52, 53), or IL-1 β mRNA in whole striatum or thalamus, and IL-1 α mRNA in hippocampus (54), although blood was not perfused from tissues in these experiments. For the most part, the time course of appearance of this IL-1 β is rather later than the CNS actions being discussed, although it may be that IL-1 can act at sites other than the hypothalamus, such as innervating noradrenergic pathways (see Quan et al. for discussion (51)). Collectively, this data suggests that IL-1 can be induced in brain glial cells and perivascular macrophages, given enough LPS in the brain circulation, but its distribution and timing do not suggest an important pathophysiological role in most circumstances. The timing and untargeted distribution also indicate that it is extremely unlikely to have been induced via neural pathways.

It is sometimes suggested that, where IL-1 can be shown to modify CNS activity, it is likely to act directly on the CNS because low doses are effective in the brain, whereas similar doses are ineffective in the periphery (6, 55). However, activity depends on concen-

tration, and since IL-1 has a plasma half-life in rats of approximately 2.5 min (56), most peripherally administered IL-1 will be cleared extremely rapidly, whereas intracerebroventricular (icv) IL-1 will be cleared more slowly. Specific antagonism of IL-1 in the brain should provide better evidence for its action and we found that fever induced by iv LPS was indeed inhibited by intracerebroventricular (icv) IL-1ra (57). Similarly icv IL-1ra can abolish depression of food intake and induction of hypothalamic IL-1 β mRNA induced by ip LPS (58). However, even in the latter experiment, the 4 μ g dose of IL-1ra would represent approximately 4000 times a biologically effective concentration of IL-1 (~100 pg/ml) if only 10% distributed to the peripheral tissue fluid. This might seem fanciful, particularly if sustained by the misconception that the BBB is significant as a barrier to substances leaving the CNS as it is to those entering. However, that this is not the case was demonstrated practically and effectively in experiments where neutralising antibodies administered icv blocked not only the central but also the peripheral actions of TNF α or neuropeptides (59, 60).

IL-6 and the CNS

Partly on the basis that there was no good relationship between fever and IL-1 in plasma, and because a better relationship was emerging with IL-6, Matt Kluger's group used IL-1 β neutralising antibodies to show that, following LPS injection, IL-1 β production was necessary both for induction of fever and for the appearance of IL-6 in plasma (14). The plasma IL-6 concentration was closely related to the fever and led them to suggest IL-6 was more likely to be the responsible circulating pyrogen. Subsequently they showed that IL-6 was an effective pyrogen when given icv (61) and that low concentrations of IL-6 could be detected in the cerebrospinal fluid (csf). Contemporaneously, Blatteis et al., demonstrated that both icv IL-6 and iv IL-6 could induce fever in guinea pigs (62), confirming previous evidence from experiments with rabbits that iv IL-6 was a pyrogen (63). Other groups also showed that IL-6 could be detected in csf, either after iv injection of LPS in cats (64), or rats

(65) or ip injection of LPS in rats (65). However, where comparisons were made, csf concentrations were observed to be one or two orders of magnitude lower than in plasma (61, 65).

In an attempt to reduce the effects of systemic LPS we employed the subcutaneous air pouch model in the rat and injected the air pouch with LPS (40). In initial experiments we used TPS in the pouch but, although it induced high fevers it had the disadvantage of making bioassay of locally produced cytokines almost impossible. The air pouch model showed that IL-1, TNF and IL-6 were produced locally, with TNF being very rapidly induced and both TNF and IL-6 being present at a high concentration. However, only IL-6 reached a detectable concentration in the plasma and this showed a very similar time course to the fever (Fig. 1 (40)). Subsequently it was shown that administration of anti-IL-6 serum abolished the fever induced by intra-pouch LPS (66), but these same experiments also showed that, although human IL-6 injected into the pouch reached high concentrations in plasma, it failed to induce fever unless a sub-pyrogenic dose of IL-1 β was co-injected.

The key role of IL-6 was supported by the observation that IL-6-deficient (IL-6 $^{-/-}$) mice failed to develop fevers in response to LPS or IL-1 β administered ip, or IL-1 β administered icv, but did respond to icv IL-6 (20). Interestingly, subsequent experiments showed IL-6 $^{-/-}$ mice could respond with a normal fever to iv LPS at a higher dose, but did not develop fe-

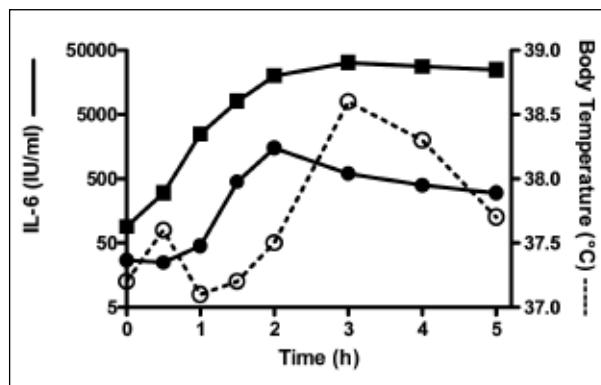


Figure 1. Kinetics of bioactive IL-6 induction in air pouch lavage fluid (○) and plasma (●) compared to thermal responses of rats (■) after intrapouch injection of 100 μ g/kg LPS (n=3 to 4 rats per time point). Adapted from Miller et al., 1997 (Ref. 40).

ver in response to intramuscular (im) TPS (13). In this same series of experiments IL- β $-/-$ mice did not develop fevers to 100 $\mu\text{g}/\text{kg}$ ip LPS or TPS but only had slightly reduced fevers with LPS at 2.5 mg/kg ip, suggesting again that LPS can mediate effects directly. The IL-6 $-/-$ mice were also resistant to fevers induced by TNF α (67).

Evidence has similarly accumulated implicating IL-6 as the circulating mediator of HPAA activation, except again where circulating LPS could be implicated as acting directly (68). Although the LPS was injected ip in these experiments, approximately 10% of LPS administered appeared in the circulation within 15 min and its effect could be reduced by the iv injection of an LPS antagonist. This antagonist did not affect IL-1 β or IL-6 production in the peritoneal cavity but reduced HPAA activation and plasma concentrations of IL-6, indicating that most IL-6 was not produced in the peritoneal cavity. This contrasts with the situation where inflammation remains localised. In experiments with Andy Turnbull, injection of TPS into a rat hind limb induced an increase in plasma IL-6 that was rapidly terminated by application of a tourniquet to the limb and the fall in IL-6 concentration indicated that it had a plasma half life of < 1.5 min (69). This rapid fall in plasma IL-6 demonstrated that IL-6 was not induced systemically to any significant extent and confirmed that IL-1 and TNF α were not present in the circulation in biologically relevant amounts since they would otherwise have sustained IL-6 production in other tissues. In this same series of experiments, administration of im TPS in mice showed that IL-1, IL-6 and TNF α were induced in the muscle, but only IL-6 was present in the plasma, which was the same pattern we had seen with the rat air pouch (40). A sustained increase in HPAA activity was apparent, but where IL-6 $-/-$ mice were used, or anti-IL-6 serum was administered, the plasma ACTH and corticosterone was significantly reduced at 8 to 18h (69), a time when the hind limb inflammation had reached its peak. Neither the early stress-induced response to TPS, nor the response to restraint stress, was affected in IL-6 $-/-$ mice, which is consistent with the observation that the early HPAA response is reduced by deafferentation of the medial basal hypothalamus or neonatal capsaicin treatment, whereas

the later phase is resistant (70). It also explains why others had suggested the HPAA was not affected by IL-6, since their examination of the HPAA was at 1.5 to 2 h, when neural afferent input is still critical (13, 44, 71). Other experiments have shown that activation of the HPAA by lower doses of LPS, and by IL-1 β , are reduced in IL-6 $-/-$ mice (72), as is the HPAA following viral infection (73). Earlier experiments had indicated an important role for IL-1 in HPAA activation, but although they demonstrated a reduction in HPAA activity following inhibition of IL-1 (73-75) or in the absence of IL-1 (76), this could not support the conclusion that IL-1 had a direct effect on the CNS. Our own experiments showed that IL-1 receptor deficient mice had a reduced HPAA response, and IL-1 β $-/-$ mice had previously been shown to be completely resistant to induction of TPS-induced fever (77), but this could be attributed to the fact that they failed to induce production of IL-6 (69). It is important to stress that the case is certainly not being made that IL-1 and TNF α are not important, or are less important in the inflammatory pathways that lead to CNS activation. Our own studies and those of others have certainly shown that this is not so (57, 67, 78). But the data do indicate that IL-6 is the important circulating mediator.

In addition to examples of CNS activation by IL-6 described above, others have shown that infusion of IL-6 can induce *c-Fos* in the hypothalamus (79) and can directly activate the HPAA (80). However, the effect of IL-6 appeared relatively weak and generally less potent than IL-1 or TNF α (80-82). Partly, this may be explained by the short half-life, but IL-1 also has a short half-life and it does not explain the lack of efficacy of the IL-6 injected into the air pouch that was mentioned above (66), because in that case the plasma concentrations were sustained for a period of hours. However, a number of other features may explain this. Firstly, IL-6 does not induce other inflammatory cytokines, whereas IL-1 and TNF are potent inducers of IL-6 (83, 84). In fact IL-6 has anti-inflammatory actions in respect of its capacity to induce IL-1ra and the soluble TNF receptor p55 (85), quite apart from its action on the HPAA. This means that whereas IL-1 and TNF provoke the induction of a variety of other tissue factors associated with inflammation (Table 1), and

these may synergise with their actions, IL-6 has little action in this respect. Enhancement of the action of IL-6 by factors produced during inflammation was hinted at above in terms of those air pouch experiments where human IL-6 was only effective if co-injected with a sub-pyrogenic dose of IL-1 β (66). A similar effect was seen in behavioural experiments, where icv IL-6 was only effective in reducing social exploration, or prolonging immobility, when a sub-effective dose of IL-1 β was co-administered, although co-administration of icv IL-1 β and IL-6 was not found necessary for provoking fever or HPAA activation (86). A possible explanation for this is the requirement for sIL-6R. When the IL-6-binding receptor (IL-6R) binds IL-6 it is not able to activate cells unless a 130 KDa glycoprotein co-receptor (gp130) is present. The gp130 is responsible for signal transduction, via phosphorylation of the signal transducers and activator of transcription, STAT 1 and STAT3, by Janus kinases (87). Unlike gp130, which is present on most cell types, IL-6R is present on few cell types, including monocytes and hepatocytes. However, as described earlier, it exists in a soluble form (sIL-6R) that can bind IL-6 without neutralising it, and distinct from the neutralizing action of soluble receptors for IL-1 and TNF. In fact the soluble IL-6:sIL-6R complex is able to bind to gp130 on cells that have no IL-6R themselves, such as endothelial cells, and addition of sIL-6R allows IL-6 to activate these cells (88). When injected icv sIL-6R was able to enhance the effect that IL-6 had in respect of inducing fever and reducing food intake and locomotor activity in rats (89). However, levels of membrane IL-6R expression can also be regulated. Glucocorticoids are able to increase expression of IL-6R in hepatocytes (90), and rats injected iv with LPS, IL-1 β , or even IL-6, express increased amounts of IL-6R in cerebral blood vessels (91, 92). Control of IL-6R expression during the inflammatory response, possibly by IL-6 itself, may therefore be a key factor in responsiveness to IL-6.

Prostaglandins

The ability of cyclooxygenase (COX) inhibitors to prevent the febrile response was understood clinically long before their mechanism of inhibiting pro-

staglandin (PG) synthesis was known, or it was shown that they could inhibit experimental fever (93). However, the first demonstration that PGE₂ caused fever when injected into the third ventricle of cats and rabbits was in 1971. The role of PGs is more difficult to dissect in the case of the HPAA, because of its actions on the pituitary and adrenal, but the consensus is clearly that there is an important action on the hypothalamus (reviewed in (2)). Similarly, although their importance in behavioural responses is less well discussed (5), the ability of the COX inhibitors, indomethacin and piroxicam (but not aspirin) to inhibit IL-1 β effects on behaviour are described (94), and those who have experienced the sickness that follows being injected with endotoxin in the cause of science can well attest the post experimental, restorative action of aspirin (personal observations!).

The role of PGs in neural afferent signalling will be considered below, but in respect of a link to circulating mediators it is necessary to consider how they might be produced to act on the CNS. One possibility is that they are produced in the periphery and circulate to the brain. Certainly the PGE₂ concentration can be shown to increase in the circulation following injection of LPS. Some early, elegant experiments in sheep showed that jugular vein infusion or intracarotid injection of LPS produced a rapid increase in carotid artery and jugular vein PGE₂, which was associated with the first phase of a biphasic fever (95). Injection of LPS into the femoral vein also produced a fever, and increased PGE₂ in the jugular and femoral veins, but PGE₂ was not detected in the carotid artery, indicating that PGE₂ was unlikely to be reaching the CNS during the fever. The importance of PGE₂ was indicated because only PGE₂, and not PGD or PGF_{2a}, induced a rise in temperature. The iv injection of LPS into rabbits gave somewhat different results, since two peaks of plasma PGE₂ were apparent, coinciding with two peaks of LPS-induced fever (96). Although this makes it clear that peripheral PGE₂ is induced, and can cause fever, it has not been entirely clear whether the concentrations induced by LPS are sufficient, or access the CNS, and doubts over the role of circulating PGs continue (see (4, 97)). Part of the objection is that the pulmonary circulation removes them very efficiently, although examination of this did

not conclude that circulating PGE₂ could not act as an endocrine hormone (98, 99). In fact an early observation that LPS could itself increase the half life of PGE₂ through the lungs has recently been lent support by experiments showing that genes controlling transport and catabolism of PGE₂ are downregulated following injection of LPS (100). Accompanying data showed increases in transcription of genes for secretory phospholipase A₂, the inducible form of COX (COX-2) and microsomal PGE synthase in the liver, lungs and hypothalamus, with the increase in COX-2 mRNA being maximal in the liver by 35 min and the hypothalamus by 95 min (101).

Whether circulating PGs are important or not, it is necessary to address what the stimulus might be for their production. The possibility to which we must return, in the context of the majority of experiments, is that LPS in the circulation acts directly on the CNS or other tissues to induce PGs. In terms of direct action on the brain, immunocytochemical staining revealed PGE₂ in brain microvasculature, particularly around the CVOs, and neuronal PGE₂ in the paraventricular and supraoptic nuclei after iv administration of a very high dose (2.5mg/kg) of LPS (102). Where LPS can access the circulation it can induce cytokines around the brain, and the pattern of IL-1 β expression after injection of LPS, particularly around blood vessels and the CVOs, has been mentioned earlier. TNF α gene expression has a very similar pattern, though again this only becomes extensive and apparent in parenchyma where very high LPS doses are used (103). A more modest dose of ip LPS induced IL-6 gene expression around the choroid plexus and the CVOs (92). So when LPS was found to induce COX-2 gene expression in the microvasculature of the rat and mouse brain it was possible that the effect was direct (104, 105). However, IL-1 β was found to induce similar COX-2 gene expression in the brain microvasculature of rats and mice, and TNF α produced more modest expression (106, 107). The im injection of TPS also induced COX-2 gene expression in brain capillaries, with a similar time course to the limb inflammation, although it was noted that administration of IL-6 did not induce COX-2 gene expression (107). This latter observation is interesting in light of a) the general comments above about inactivity of IL-6 when given

alone; b) the demonstration that even peripheral responses to TPS are IL-6-dependent (44); c) we found immunostaining of COX-2 in brain blood vessels of normal but not IL-6 $-/-$ mice injected im with TPS (69); d) this group had themselves shown the IL-6R could be induced by IL-6 and other inflammatory stimuli, and had suggested that this might be necessary before IL-6 could act (92).

In addition to COX-2, brain endothelium expresses microsomal PGE synthase (mPGES), the enzyme that converts PGH₂ to PGE after COX-2 converts arachidonate to PGH₂. Like COX-2, mPGES is not expressed constitutively, but is expressed following injection of IL-1 β and in animals with adjuvant-induced arthritis (108, 109). The importance of this pathway is highlighted by the fact that animals deficient in mPGES do not develop fever after injection of LPS, although they do when PGE₂ is administered centrally (110). Once in the brain PGE₂ can potentially bind a variety of receptor types, EP₁ to EP₄, but the key target for fever appears to be EP₃, since mice lacking this receptor, but not the others, fail to develop fevers in response to PGE₂, IL-1 β or LPS (22).

Neural input revisited

One of the conundrums that confront analysis of results in this area is the extreme rapidity with which the CNS responds to inflammation. In the case of the HPAA the case is probably clearest, because of the undisputed importance of well described neural pathways at the early stage of responses, and in this respect it is somewhat puzzling that the role of cytokine-mediated input to this response has been evaluated at a time that neural input can be expected to be dominant (13, 44, 71). Aside from CNS responses that impact on peripheral immune regulation, and have even more protracted kinetics, the more difficult responses to tease apart are behavioural and fever responses. In respect of fever the difficulties of explaining responses in terms of hormonal mediation are well described by Clark Blatteis (4, 97). In essence, the principal difficulty is that fever is often observed well before there could be significant induction of cytoki-

nes or COX-2, either in the periphery or the CNS. Since neurally mediated activation seems the only mechanism fast enough to account for this, a number of investigations have explored this possibility, with variable results.

The main target of investigation has been the vagus. Consistent with the hypothalamus being the main target for activation by inflammatory stimuli, Wan et al., showed that icv and ip LPS increased the number of c-Fos stained neurons in the paraventricular nucleus and that increase in plasma corticosterone concentration followed a similar pattern (111). In subsequent experiments they showed that c-Fos induction by LPS was inhibited by both indomethacin and the glutamate N-methyl-D-aspartate receptor antagonist MK801, although only MK801 inhibited c-Fos induction by foot shock (112). When these animals were subject to subdiaphragmatic vagotomy (SVGX) this reduced c-Fos induction that followed administration of ip LPS, but it had no effect if the LPS was given iv, and increased the c-Fos after foot shock, suggesting that circulating LPS activated the CNS via another route and other neural afferent pathways are able to activate the hypothalamus after vagotomy. Reduction of the corticosterone response, following SVGX but not hepatic vagotomy (HVGX), was also shown in response to an ip challenge with IL-1 β and was accompanied by an inhibition of the hypothalamic noradrenaline depletion that normally accompanies an HPA axis response (113). Similar results were shown in experiments where c-Fos expression, induced by a low dose of ip LPS, was reduced by SVGX but not HVGX (114). A reduction in the plasma ACTH response accompanied the SVGX effect on c-Fos, although the ACTH response to a higher dose of LPS was less affected and plasma corticosterone was not affected by SVGX after either LPS dose.

The first vagotomy experiments with fever are perhaps most remarkable by virtue of the huge stress-induced hyperthermia apparent prior to ip injection of IL-1 β (15). SVGX reduced fever attributable to IL-1 β injection, but also abolished the stress-induced fever. In contrast, administration of IL-1ra abolished only the IL-1 β -induced phase of fever. As with the HPA axis, HVGX was without effect on either element of the temperature increase. In guinea pigs

SVGX was shown to reduce the febrile response to ip LPS and this was accompanied by a reduction in PGE₂ recovered from the anterior hypothalamus by microdialysis (115). Similar results were obtained by Goldbach et al., when the LPS was given ip, but fever induced by im LPS was not affected (116). In respect of behavioural responses, SVGX attenuated the reduction in activity of rats observed after ip injection of LPS and it also appeared to reduce the expression of IL-1 β mRNA in the brain (117). In further experiments, social interaction was employed as a behavioural measure and was assessed 2 weeks after the same rats had been used to examine their fever response to LPS (118). Although SVGX had not had an impact on the fever response, the depressed social interactions caused by LPS were reversed. In a rare study of the effect of vagotomy in mice, the effect of SVGX on both behaviour and the HPA axis was examined (119). Behaviour was assessed in terms of food intake, sweetened milk intake and locomotor activity, which were all reduced by ip IL-1 β and LPS. SVGX slightly attenuated the response to IL-1 β , but had no effect on responses to LPS. In contrast, the plasma corticosterone and ACTH increases induced by ip IL-1 β were not significantly affected by SVGX, although the increase induced by LPS was attenuated. The picture is therefore not entirely clear, and would perhaps be helped by some dose response studies, but deafferentation of the vagus (in fact efferents are removed as well) certainly can reduce responses, particularly where lower doses of LPS or IL-1 β are administered ip.

Apart from its important role in conveying information from the viscera, the vagus has the advantage of being relatively easy to dissect, but the question arises as to whether it is a special case. To test this some interesting experiments were done with a guinea pig air pouch model. Teflon chambers attached to catheters were implanted in the pouches, allowing subsequent administration of LPS together with local anaesthetic. When a high dose of LPS was administered the anaesthetic had no effect on the induced fever, but when a lower dose was administered it reduced the fever, indicating a contribution to the fever from neural afferents (120). The increase in plasma IL-6 was shown not to be affected in these animals and control experiments showed that contralateral administration

of local anaesthetic did not have the same effect as when placed in the chamber with the LPS. Transection of the glossopharyngeal nerve has also been shown to reduce fever resulting from injection of IL-1 β or LPS into soft palate (121), so the vagus is not unique in its contribution to CNS input following an inflammatory stimulus.

Activating the neural afferents

Although the neural afferents present a rapid means to activate the CNS, brisk CNS responses to inflammation require rapid induction of mediators to excite the afferent nerve terminals. Induction of cytokines would generally seem too slow to explain the kinetics apparent with many fevers and an intriguing mechanism has been suggested by a series of experiments from the laboratory of Clark Blatteis (4, 41). Depletion of complement in guinea pigs and the use of complement-deficient mice has indicated that direct activation of the alternate complement pathway by LPS could result in rapid production of C5a fragments that are then able to induce production of PGE₂ by constitutive COX-1 (21, 24, 122, 123). This is an extremely plausible explanation, although the question arises as to whether other factors may be rapidly activated to stimulate neural afferent pathways. For instance, Hageman factor (Factor XII) is also activated by LPS and bacterial cell surfaces and can induce kinins such as bradykinin, via activation of prekallikrein (124, 125). However, although icv injection of bradykinin inhibitors has been shown to inhibit fever induced by icv or iv LPS (126, 127), there appear to be no investigations of the role of peripheral bradykinin on fever induced by peripheral inflammation. This is interesting because bradykinin is an important mediator of pain and inflammation (128). In addition to being a potent inducer of PGs, via both COX-1 and COX-2, it stimulates IL-1 and TNF release from macrophages, as well as IL-6 from fibroblasts and other cells (129, 130). In relation to its role in the hyperalgesic effect of cytokines it was found to exert its action by initiating cytokine release via a partially COX-dependent mechanism (131). However, the ability of bradykinin inhibitors to prevent LPS-in-

duced hyperalgesia seems ambiguous, having inhibited hyperalgesia in one set of experiments (131), but not subsequently (132). Bradykinin appeared to play a particularly important role in inducing TNF α during the hyperalgesic response (133), which is interesting in view of the speed with which TNF α is released following LPS injection into a subcutaneous air pouch (40).

Pursuing the idea that vagal neural afferents contribute towards activating the CNS after injection of LPS, Székely et al. attempted to remove input from neural afferents of the vagus by ip injection of capsaicin (133). This removed only the early phase of a triphasic fever induced by ip LPS, whereas vagotomy attenuated only the last phase, which does not really fit with the vagus being essential to the early phase of fever. Subsequent experiments to determine the mechanism of action of capsaicin indicated that it was in fact probably acting via a non-neural mechanism (134), and these authors speculated that the capsaicin may interfere with PGE₂ induction by non-neural cells, which brings us back to question the importance of PGs.

Neural integration and sensitisation

However the peripheral signals reach the brain, they have to be integrated at the appropriate level. In terms of neural pathways, the noradrenergic system clearly has an important role to play, particularly in respect of fever and the HPAA. Noradrenergic pathways to the areas of the hypothalamus associated with these responses have been mapped (135) and the role of noradrenergic pathways and receptors is discussed elsewhere (136, 137). With respect to sickness behaviour, the main focus other than the vagal input discussed has been in the context of pain (28). However, PGs appear to play an important role in both of these neural pathways, (138, 139) and there is evidence that IL-6 may do so as well (140, 141).

In the absence of an inflammatory stimulus the distribution of both COX-1 and COX-2 is principally neuronal, rather than glial. COX-1 is found particularly in areas of the forebrain involved in integrative functions and autonomic regulation (142). COX-2

is distributed rather differently, in the cortex, hippocampus, amygdala, the paraventricular nucleus, around the OVL and in the brainstem, indicating a role in visceral and other sensory pathways associated with autonomic, behavioural and endocrine responses. Unlike COX-2 in the periphery, or in the brain microvasculature, COX-2 in brain neurons is constitutive and seems to be maintained by synaptic activity (143, 144).

Within the CNS PGs appear to increase transmission by inducing release of presynaptic mediators and by direct activation of postsynaptic neurons (145). In sensory afferent pathways the relative importance of PGs in the periphery and CNS remains unclear and this may be because it is a dynamic process. For example, where inflammation was induced in a knee joint by injection of kaolin, application of PGE₂ to the spinal cord facilitated neuronal activity, prior to and during development of the inflammation, and facilitated dorsal horn neuronal firing by N-methyl-D-aspartate (146). This response could be inhibited by application of indomethacin to the spinal cord, suggesting that PGs had a physiological role in the response. However, once the inflammation had developed, the spinal cord responses were only reduced when the indomethacin was given systemically, and not topically, signifying that maintenance of sensitisation was a peripheral function of PGs. In peripheral tissues PGs seem to sensitize neurons by decreasing the activation threshold of sodium channels and increasing sensitivity to activation by mediators such as bradykinin (145) and, in general terms PGs alter the sensitivity of neurons, rather than act as direct neurotransmitters; usually increasing responsiveness, but not always. For instance, PGE₂ increases the firing rate of temperature insensitive neurons in the preoptic area of the hypothalamus but reduces firing rates in warm-sensitive neurones (147).

Synthesis

In considering the data derived from analysing these CNS responses the variability is striking, even for the same basic response. It is sometimes considered that work with experimental animals is easier than

in humans, because one cage of rats or mice is much the same as another, unlike patients or other human subjects. Within a laboratory this may be true, but it is clearly often not the case between laboratories. However, it is possible to see some pattern through the variability, if filtered by a certain degree of prejudice. The main prejudice is in respect of the use of systemic LPS, since it is generally impossible to tell where it is acting directly. This therefore forms the starting point for considering how the various data sets may be interpreted and brought together (Fig. 2). When administered *iv*, or *ip* at high doses, LPS seems to pretty much hit every target and can directly impact on the CNS. It was partly this problem of not knowing what the LPS was doing that led us to deploy the air pouch as a means of restricting the inflammatory insult, whilst use of LPS in the pouch allowed some common features with the mainstream experiments in the field (40). The principal observations with this model were the very rapid increase of cytokines in the pouch, particularly TNF, the absence of IL-1 and TNF in the plasma, and the way that increase in fever followed the rise in plasma IL-6 about 30 min to 1h later (Fig. 1). This, combined with the repeated failure to detect biologically active IL-1 in plasma, in a variety of situations and despite having devised a sensitive assay specifically for this purpose (148, 149), made it almost impossible to take seriously the idea of IL-1 as a circulating mediator. IL-1 and TNF are clearly important inflammatory mediators, as confirmed by so many experimental observations and the development of potent anti-IL-1 and anti-TNF α therapies for rheumatoid arthritis and other inflammatory disease, but unlike IL-6 they make no sense as endocrine hormones.

In terms of inflammation-linked factors conveying hormonal information to the CNS, the first identifiable element is therefore IL-6 (Fig. 2). With the exception of still needing to resolve some issues about factors with which it synergises to produce its effect, such as sIL-6R or induced membrane IL-6R, the data cited above clearly give it a key role. A further common feature of the CNS responses under discussion is that they can each be induced via strictly neural pathways, in the absence of inflammation. The neural input to the various responses certainly varies,

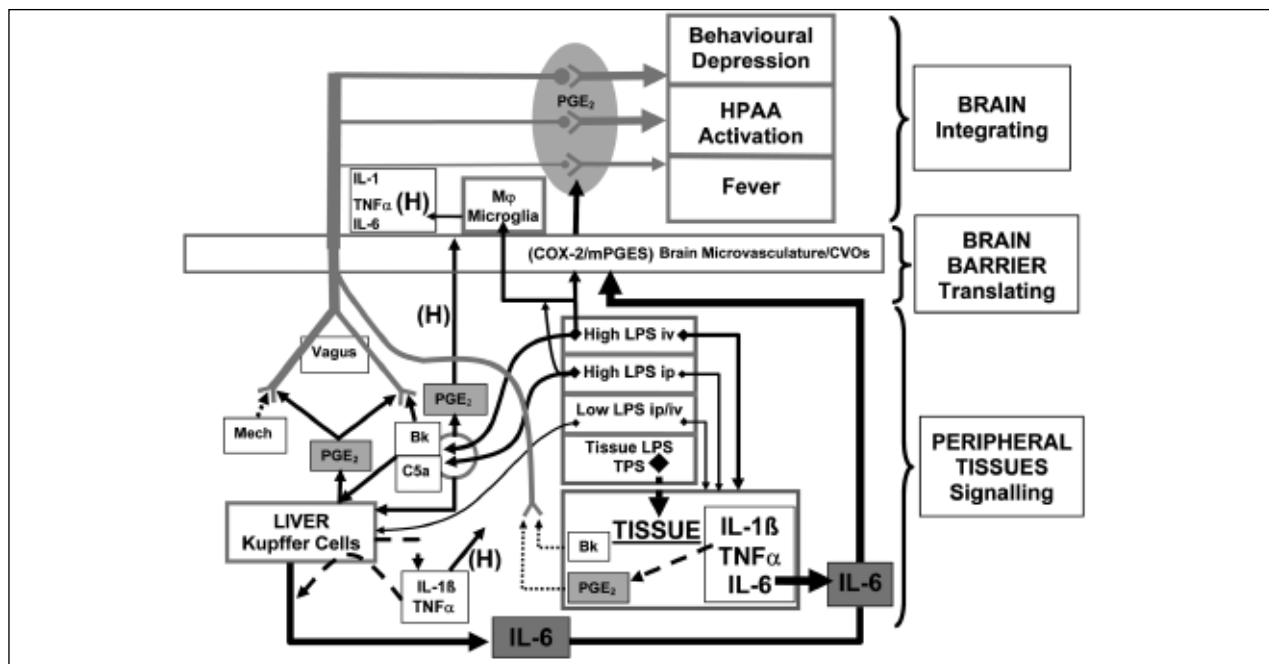


Figure 2. Scheme suggesting how the CNS may recognise peripheral inflammation. The starting point is the central box with decreasing systemic impact at lower levels. Solid lines represent factors present in the circulation. Dashed lines represent actions restricted within individual tissues. Grey lines represent neural input. Line thickness represents relative importance of the stimulus for that factor; more and heavier arrows resulting in greater activation.

Bk = Bradykinin; C5a = Activated complement fragment 5a; COX-2 = Cyclooxygenase-2; CRH = Corticotropin releasing hormone; ip = intraperitoneal; iv = intravenous; Mech. = Mechanical or other efferent stimulus to the non-hepatic visceral branches of the vagus; PGE₂ = Prostaglandin E₂; M ϕ = Perivascular macrophage; mPGES = Microsomal prostaglandin E synthase; TPS = Turpentine; H = indicates that this pathway is probably only important with high doses of systemic LPS.

but it is able to work alone or together with humoral signals. Where neural afferent input is a major factor the role of IL-6 may be less important, since cytokines such as IL-1 and TNF α play an important role in peripheral tissues, possibly explaining the apparent greater dependence of behavioural responses on vagal input. Finally, but not least, there are the PGs, and PGE₂ in particular. Although vascular distribution of LPS in many experiments complicates interpretation, the data would broadly seem to support its role as a circulating mediator only when relatively large doses of LPS are used. In this situation PGE₂ may be induced in sufficient amounts, possibly via activation of complement, and directly impact on the CNS, which could account for the early phase of fever. However, experiments still need to be done to identify whether induced plasma concentrations are actually sufficient to do this. Large amounts of LPS in the peritoneal ca-

vity may also induce PGE₂, rapidly, but this probably acts only locally on neural afferents, such as those of the vagus. Whatever the role of PGs in the periphery, it is clear that PGs can modify responses within the CNS and there is now mounting evidence documenting induction of PGE₂ at the level of the brain microvasculature. Aside from LPS, the most likely candidate for initiating this during an inflammatory response is IL-6. Neural input is good at localising events in tissues, but without common neural elements in every tissue the CNS would have a hard time keeping track of inflammation. In order to do this it makes sense for inflamed tissues to send information about the burden of inflammation to the CNS, so that it can play its part in host defence. After all, if the liver can depend on the endocrine role of IL-6 to tell it how to regulate its response to inflammation, why not the brain?

Acknowledgments

I should like to thank the various individuals that I have had the privilege to work with in this area, and be named alongside in some of the papers cited here, particularly Nancy Rothwell for her continued collaboration. I hope they will not be offended by anything in this review, to which they contributed in no small part over the years.

References

- Luheshi G, Rothwell N. Cytokines and fever. *Int Arch Allergy Immunol* 1996; 109: 301-7.
- Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* 1999; 79: 1-71.
- Roth J, de Souza GEP. Fever induction pathways: evidence from responses to systemic or local cytokine formation. *Braz J Med Biol Res* 2001; 34: 301-14.
- Blatteis CM, Li SX, Li ZH, Feleder C, Perlik V. Cytokines, PGE(2) and endotoxic fever: a re-assessment. *Prostaglandin Lipid Mediat* 2005; 76: 1-18.
- Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci* 2002; 25: 154-9.
- Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol* 2004; 500: 399-411.
- Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve - An integrative interface between two supersystems: The brain and the immune system. *Pharmacol Rev* 2000; 52: 595-638.
- Pavlov VA, Tracey KJ. Neural regulators of innate immune responses and inflammation. *Cell Mol Life Sci* 2004; 61: 2322-31.
- Webster JI, Sternberg EM. Role of the hypothalamic-pituitary-adrenal axis, glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial and viral products. *J Endocrinol* 2004; 181: 207-21.
- Rivest S. Molecular insights on the cerebral innate immune system. *Brain Behav Immunity* 2003; 17: 13-9.
- Cooper AL, Rothwell NJ. Mechanisms of early and late hypermetabolism and fever after localized tissue injury in rats. *Am J Physiol (Endocrinol Metab)* 1991; 261: E698-705.
- Cooper AL, Horan MA, Little RA, Rothwell NJ. Metabolic and febrile responses to typhoid vaccine in humans - effect of beta-adrenergic-blockade. *J Appl Physiol* 1992; 72: 2322-8.
- Kozak W, Kluger MJ, Soszynski D, Conn CA, Rudolph K, Zheng H. IL-6 and IL-1 beta in fever - Studies using cytokine-deficient (knockout) mice. *Ann NY Acad Sci* 1998; 856: 33-47.
- LeMay LG, Otterness IG, Vander AJ, Kluger MJ. In vivo evidence that the rise in plasma IL 6 following injection of a fever-inducing dose of LPS is mediated by IL-1. *Cytokine* 1990; 2: 199-204.
- Watkins LR, Goehler LE, Relton JK, et al. Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 1995; 183: 27-31.
- Luheshi G, Miller AJ, Brouwer S, Dascombe MJ, Rothwell NJ, Hopkins SJ. Interleukin-1 receptor antagonist inhibits endotoxin fever and systemic interleukin-6 induction in the rat. *Am J Physiol (Endocrinol Metab)* 1996; 270: E91-5.
- Scammell TE, Griffin JD, Elmquist JK, Saper CB. Microinjection of a cyclooxygenase inhibitor into the anteroventral preoptic region attenuates LPS fever. *Am J Physiol-Regul Integr Comp Physiol* 1998; 43:R783-9.
- Szekely M, Balasko M, Kulchitsky VA, Simons CT, Ivanov AI, Romanovsky AA. Multiple neural mechanisms of fever. *Auton Neurosci-Basic Clin* 2000; 85: 78-82.
- Coelho MM, Souza GEP, Pela IR. Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1992; 263 (2 Part 2): R423-7.
- Chai Z, Gatti S, Toniatti C, Poli V, Bartfai T. Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever responses to lipopolysaccharide or IL-1 β : a study on IL-6-deficient mice. *J Exp Med* 1996; 183: 311-6.
- Li S, Holers VM, Boackle SA, Blatteis CM. Modulation of mouse endotoxic fever by complement. *Infect Immunity* 2002; 70: 2519-25.
- Ushikubi F, Segi E, Sugimoto Y, et al. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 1998; 395: 281-4.
- Roth J, Conn CA, Kluger MJ, Zeisberger E. Kinetics of systemic and intrahypothalamic IL-6 and tumor necrosis factor during endotoxin fever in guinea pigs. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1993; 265: R653-8.
- Sehic E, Li S, Ungar AL, Blatteis CM. Complement reduction impairs the febrile response of guinea pigs to endotoxin. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1998; 43: R1594-603.
- Revhaug A, Michie HR, Manson JM, et al. Inhibition of cyclo-oxygenase attenuates the metabolic response to endotoxin in humans. *Arch Surg* 1988; 123: 162-70.
- Soop M, Duxbury H, Agwunobi AO, et al. Euglycemic hyperinsulinemia augments the cytokine and endocrine responses to endotoxin in humans. *Am J Physiol (Endocrinol Metab)* 2002; 282: E1276-85.
- Oka T, Oka K, Hori T. Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosom Med* 2001; 63: 476-86.
- Watkins LR, Maier SF. The pain of being sick: Implications of immune-to-brain communication for understanding pain. *Annu Rev Psychol* 2000; 51: 29-57.
- Dantzer R, Konsman JP, Bluthé RM, Kelley KW. Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? *Auton Neurosci-Basic Clin* 2000; 85: 60-5.
- Besedovsky H, Sorkin E, Keller M, Muller J. Changes in blood hormone levels during immune-response. *Proc Soc Exp Biol Med* 1975; 150: 466-70.

31. Besedovsky H, Sorkin E, Felix D, Haas H. Hypothalamic changes during immune-response. *Eur J Immunol* 1977; 7: 323-5.
32. Besedovsky HO, Delrey AE, Sorkin E. What do the immune-system and the brain know about each other. *Immunol Today* 1983; 4: 342-6.
33. Berkenbosch F, van Oers J, del Rey A, Tilders F, Besedovsky H. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science* 1987; 238: 524-6.
34. Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W. Interleukin-1 stimulates the secretion of hypothalamic corticotrophin-releasing factor. *Science* 1987; 238: 522-4.
35. Medvei VC. A history of endocrinology. Lancaster; MTP Press Ltd, 1982: 7-8.
36. Ibelgauts H. Cope with cytokines. <http://www.copewith-cytokines.de/cope.cgi>.
37. Hopkins SJ. The pathophysiological role of cytokines. *Legal Med* 2003; 5: S45-57.
38. Hirano T. Interleukin 6 and its receptor: ten years later. *Intern Rev Immunol* 1998; 16: 249-84.
39. Long NC, Otterness I, Kunkel SL, Vander AJ, Kluger MJ. Roles of interleukin-1-beta and tumor necrosis factor in lipopolysaccharide fever in rats. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1990; 259: R724-8.
40. Miller AJ, Luheshi G, Rothwell NJ, Hopkins SJ. Local cytokine induction by lipopolysaccharide in the rat air pouch and its relationship to the febrile response. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1997; 272: R857-61.
41. Blatteis CM, Li SX, Li ZH, Perlik V, Feleder C. Complement is required for the induction of endotoxic fever in guinea pigs and mice. *J Therm Biol* 2004; 29: 369-81.
42. Beutler B. Inferences, questions and possibilities in toll-like receptor signalling. *Nature* 2004; 430: 257-63.
43. Castell JV, Andus T, Geiger T, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in rat and men. In: Spreafico F, Austen KF, Hadden JW, Yamamya Y eds. *Advances in Immunopharmacology*. Pergamon Press, Oxford, UK, 1989: 191-201.
44. Fattori E, Cappelletti M, Costa P, et al. Defective inflammatory response in interleukin 6-deficient mice. *J Exp Med* 1994; 180: 1243-50.
45. Guilan D, Baker TJ, Shih L-CN, Lachman LB. Interleukin 1 of the central nervous system is produced by amoeboid microglia. *J Exp Med* 1986; 164: 594-604.
46. Woodrooffe MN, Sarna GS, Wadhwa M, et al. Detection of interleukin-1 and interleukin-6 in adult rat brain, following mechanical injury, by *in vivo* microdialysis - evidence of a role for microglia in cytokine production. *J Neuroimmunol* 1991; 33: 227-36.
47. van Dam A-M, Brouns M, Louisse S, Berkenbosch F. Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats - a pathway for the induction of non-specific symptoms of sickness. *Brain Res* 1992; 588: 291-6.
48. Banks WA, Kastin AJ, Durham DA. Bidirectional transport of interleukin-1 alpha across the blood brain barrier. *Brain Res Bull* 1989; 23: 433-7.
49. Gutierrez EG, Banks WA, Kastin AJ. Murine tumor necrosis factor-alpha is transported from blood to brain in the mouse. *J Neuroimmunol* 1993; 47: 169-76.
50. Vandam AM, Bauer J, Tilders FJH, Berkenbosch F. Endotoxin-induced appearance of immunoreactive interleukin-1-beta in ramified microglia in rat-brain - a light and electron-microscopic study. *Neurosci* 1995; 65: 815-26.
51. Quan N, Sundar SK, Weiss JM. Induction of interleukin 1 in various brain regions after peripheral and central injections of lipopolysaccharide. *J Neuroimmunol* 1994; 49: 125-34.
52. Hillhouse EW, Mosley K. Peripheral endotoxin induces hypothalamic immunoreactive interleukin-1 β in the rat. *Brit J Pharmacol* 1993; 109: 289-90.
53. Layé S, Parnet P, Goujon E, Dantzer R. Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. *Mol Brain Res* 1994; 27: 157-62.
54. Ban E, Haour F, Lenstra R. Brain interleukin-1 gene-expression induced by peripheral lipopolysaccharide administration. *Cytokine* 1992; 4: 48-54.
55. Rothwell NJ. Mechanisms of the pyrogenic actions of cytokines. *Eur Cytokine Net* 1990; 1: 211-3.
56. Poole S, Bird TA, Selkirk S, et al. Fate of injected interleukin 1 in rats: sequestration and degradation in the kidney. *Cytokine* 1990; 2: 416-22.
57. Miller AJ, Hopkins SJ, Luheshi G. Sites of action of IL-1 in the development of fever and cytokine responses to tissue inflammation in the rat. *Brit J Pharmacol* 1997; 120: 1274-9.
58. Kent S, Bluthe RM, Dantzer R, et al. Different receptor mechanisms mediate the pyrogenic and behavioral-effects of interleukin-1. *Proc Natl Acad Sci USA* 1992; 89: 9117-20.
59. Turnbull AV, Rivier CL. Intracerebroventricular passive immunization. I. The effect of intracerebroventricular administration of an antiserum to tumor necrosis factor-alpha on the plasma adrenocorticotropin response to lipopolysaccharide in rats. *Endocrinology* 1998; 139: 119-27.
60. Turnbull AV, Rivier CL. Intracerebroventricular passive immunization. II. Intracerebroventricular infusion of neuropeptide antisera can inhibit neuropeptide signaling in peripheral tissues. *Endocrinology* 1998; 139: 128-36.
61. LeMay LG, Vander AJ, Kluger MJ. Role of interleukin 6 in fever in rats. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1990; 258: R798-803.
62. Blatteis CM, Quan N, Xin L, Ungar AL. Neuromodulation of acute-phase responses to interleukin-6 in guinea pigs. *Brain Res Bull* 1990; 25: 895-901.
63. Helle M, Brakenhoff JPJ, De Groot ER, Aarden LA. Interleukin 6 is involved in interleukin 1-induced activities. *Eur J Immunol* 1988; 18: 957-9.
64. Cocceani F, Lees J, Mancilla J, Belizario J, Dinarello CA. Interleukin-6 and tumor necrosis factor in cerebrospinal fluid: Changes during pyrogen fever. *Brain Res* 1993; 612: 165-71.
65. Coelho MM, Luheshi G, Hopkins SJ, Pela IR, Rothwell NJ. Multiple mechanisms mediate the antipyretic action of

- glucocorticoids. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1995; 269: R527-35.
66. Cartmell T, Poole S, Turnbull AV, Rothwell NJ, Luheshi GN. Circulating interleukin-6 mediates the febrile response to localised inflammation in rats. *J Physiol-London* 2000; 526: 653-61.
 67. Sundgren-Andersson AK, Ostlund P, Bartfai T. IL-6 is essential in TNF-alpha-induced fever. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1998; 44: R2028-34.
 68. Lenczowski MJP, van Dam A-M, Poole S, Larrick JW, Tilders FJH. Role of circulating endotoxin and interleukin-6 in the ACTH and corticosterone response to intraperitoneal LPS. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1997; 273: R1870-7.
 69. Turnbull AV, Prehar S, Kennedy AR, Little RA, Hopkins SJ. Interleukin-6 is an afferent signal to the hypothalamo-pituitary-adrenal axis during local inflammation in mice. *Endocrinology* 2003; 144: 1894-906.
 70. Turnbull AV, Dow RC, Hopkins SJ, White A, Fink G, Rothwell NJ. Mechanisms of activation of the pituitary-adrenal axis by tissue injury in the rat. *Psychoendocrinol* 1994; 3: 165-70.
 71. Fantuzzi G, Dinarello CA. The inflammatory response in interleukin-1-deficient mice: comparison with other cytokine-related knock-out mice. *J Leukoc Biol* 1996; 59: 489-93.
 72. Wang JP, Dunn AJ. The role of interleukin-6 in the activation of the hypothalamo-pituitary-adrenocortical axis and brain indoleamines by endotoxin and interleukin-1 beta. *Brain Res* 1999; 815: 337-48.
 73. Ruzek MC, Miller AH, Opal SM, Pearce BD, Biron CA. Characterization of early cytokine responses and an interleukin (IL)-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. *J Exp Med* 1997; 185: 1185-92.
 74. Rivier C, Chizzonite R, Vale W. In the mouse, the activation of the hypothalamic pituitary adrenal axis by lipopolysaccharide (endotoxin) is mediated through interleukin-1. *Endocrinology* 1989; 126: 2800-5.
 75. Dunn AJ, Vickers SL. Neurochemical and neuroendocrine responses to Newcastle disease virus administration in mice. *Brain Res* 1994; 645: 103-12.
 76. Horai R, Asano M, Sudo K, et al. Production of mice deficient in genes for interleukin (IL)-1 α , IL-1 β , IL-1 α/β , and IL-1 receptor antagonist shows that IL-1, is crucial in turpentine-induced fever development and glucocorticoid secretion. *J Exp Med* 1998; 187: 1463-75.
 77. Zheng H, Fletcher D, Kozak W, et al. Resistance to fever induction and impaired acute phase response in interleukin-1, deficient mice. *Immunity* 1995; 3: 9-19.
 78. Cooper AL, Brouwer S, Hopkins SJ, Luheshi GN, Turnbull AV, Rothwell NJ. Tumour necrosis factor- and fever after peripheral inflammation in the rat. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1994; 36: R1431-6.
 79. Niimi M, Wada Y, Sato M, Takahara J, Kawanishi K. Effect of continuous intravenous injection of interleukin-6 and pretreatment with cyclooxygenase inhibitor on brain *c-fos* expression in the rat. *Neuroendocrinology* 1997; 66: 47-53.
 80. Wang JP, Dunn AJ. Mouse interleukin-6 stimulates the HPA axis and increases brain tryptophan and serotonin metabolism. *Neurochem Int* 1998; 33: 143-54.
 81. Besedovsky HO, Del Ray A, Furukawa H, Ardit GM, Kabiersch A. Cytokines as modulators of the hypothalamus-pituitary-adrenal axis. *J Steroid Biochem Mol Biol* 1991; 40: 613-8.
 82. Van der Meer MJM, Sweep CGJ, Rijnkels CEM, et al. Acute stimulation of the hypothalamic-pituitary-adrenal axis by IL-1 β , TNF α and IL-6: a dose response study. *J Endocrinol Invest* 1996; 19: 175-82.
 83. Zhang Y, Lin J-X, Vilcek J. Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of a nuclear factor binding to a kB-like sequence. *Mol Cell Biol* 1990; 10: 3818-23.
 84. Libert C, Brouckaert P, Shaw A, Fiers W. Induction of interleukin-6 by human and murine recombinant interleukin-1 in mice. *Eur J Immunol* 1990; 20: 691-4.
 85. Tilg H, Trehu E, Atkins MB, Dinarello CA, Mier JW. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood* 1994; 83: 113-8.
 86. Lenczowski MJP, Bluth RM, Roth J, et al. Central administration of rat IL-6 induces HPA activation and fever but not sickness behavior in rats. *Am J Physiol (Regul Integr Comp Physiol)* 1999; 276: R652-8.
 87. Kallen KJ. The role of transsignalling via the agonistic soluble IL-6 receptor in human diseases. *Biochim Biophys Acta-Mol Cell Res* 2002; 1592: 323-43.
 88. Romano M, Sironi M, Toniatti C, et al. Role of IL-6 and its soluble receptor in induction of chemokines and leucocyte recruitment. *Immunity* 1997; 6: 315-25.
 89. Schöbitz B, Pezeshki G, Pohl T, et al. Soluble interleukin-6 (IL-6) receptor augments central effects of IL-6 in vivo. *FASEB J* 1995; 9: 659-64.
 90. Fischer CP, Bode BP, Takahashi K, Tanabe KK, Souba WW. Glucocorticoid-dependent induction of interleukin-6 receptor expression in human hepatocytes facilitates interleukin-6 stimulation of amino acid transport. *Ann Surg* 1996; 223: 610-8.
 91. Vallières L, Rivest S. Regulation of the genes encoding interleukin-6, its receptor, and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1 beta. *J Neurochem* 1997; 69: 1668-83.
 92. Vallières L, Rivest S. Interleukin-6 is a needed proinflammatory cytokine in the prolonged neural activity and transcriptional activation of corticotropin-releasing factor during endotoxemia. *Endocrinology* 1999; 140: 3890-903.
 93. Feldberg W, Saxena PN. Prostaglandins, endotoxin and lipid A on body temperature in rats. *J Physiol - London* 1975; 249: 601-15.
 94. Crestani F, Seguy F, Dantzer R. Behavioral-effects of peripherally injected interleukin-1 -role of prostaglandins. *Brain Res* 1991; 542: 330-5.

95. Skarnes RC, Brown SK, Hull SS, McCracken JA. Role of prostaglandin E in the biphasic fever response to endotoxin. *J Exp Med* 1981; 154: 1212-24.
96. Rotondo D, Abul HT, Milton AS, Davidson J. Pyrogenic immunomodulators increase the level of prostaglandin E₂ in the blood simultaneously with the onset of fever. *Eur J Pharmacol* 1988; 154:145-52.
97. Blatteis CM. The cytokine-prostaglandin cascade in fever production: fact or fancy? *J Therm Biol* 2004; 29: 359-68.
98. Robertson RP. Differential *in vivo* pulmonary degradation of prostaglandins E₁, B₁, and A₁. *Am J Physiol* 1975; 228: 68-70.
99. Eling TE, Ally AI. Pulmonary biosynthesis and metabolism of prostaglandins and related substances. *Environ Health Perspect* 1984; 55: 159-68.
100. Ivanov AI, Scheck AC, Romanovsky AA. Expression of genes controlling transport and catabolism of prostaglandin E₂ in lipopolysaccharide fever. *Am J Physiol (Regul Integr Comp Physiol)* 2003; 284: R698-706.
101. Ivanov AI, Pero RS, Scheck AC, Romanovsky AA. Prostaglandin E₂-synthesizing enzymes in fever: differential transcriptional regulation. *Am J Physiol (Regul Integr Comp Physiol)* 2002; 283: R1104-17.
102. Van Dam A-M, Brouns M, Man-A-Hing W, Berkenbosch F. Immunocytochemical detection of prostaglandin E₂ in microvasculature and in neurons of rat brain after administration of bacterial endotoxin. *Brain Res* 1993; 613: 331-6.
103. Nadeau S, Rivest S. Regulation of the gene encoding tumor necrosis factor α (TNF- α) in the rat brain and pituitary in response to different models of systemic immune challenge. *J Neuropathol Exp Neurol* 1999; 58: 61-77.
104. Cao CY, Matsumura K, Yamagata K, Watanabe Y. Induction by lipopolysaccharide of cyclooxygenase-2 messenger-RNA in rat brain - its possible role in the febrile response. *Brain Res* 1995; 697: 187-96.
105. Breder CD, Saper CB. Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide. *Brain Res* 1996; 713: 64-9.
106. Cao CY, Matsumura K, Yamagata K, Watanabe Y. Endothelial cells of the rat brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1 beta: A possible site of prostaglandin synthesis responsible for fever. *Brain Res* 1996; 733: 263-72.
107. Lacroix S, Rivest S. Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. *J Neurochem* 1998; 70: 452-66.
108. Ek M, Engblom D, Saha S, Blomqvist A, Jakobsson PJ, Ericsson-Dahlstrand A. Inflammatory response - pathway across the blood-brain barrier. *Nature* 2001; 410: 430-1.
109. Engblom D, Ek M, Andersson IM, et al. Induction of microsomal prostaglandin E synthase in the rat brain endothelium and parenchyma in adjuvant-induced arthritis. *J Comp Neurol* 2002; 452: 205-14.
110. Engblom D, Saha S, Engstrom L, et al. Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. *Nat Neurosci* 2003; 6: 1137-8.
111. Wan WH, Janz L, Vriend CY, Sorensen CM, Greenberg AH, Nance DM. Differential induction of C-fos immunoreactivity in hypothalamus and brain-stem nuclei following central and peripheral administration of endotoxin. *Brain Res Bull* 1993; 32: 581-7.
112. Wan W, Wetmore L, Sorensen CM, Greenberg AH, Nance DM. Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in rat brain. *Brain Res Bull* 1994; 34: 7-14.
113. Fleshner M, Goehler LE, Hermann J, Relton JK, Maier SF, Watkins LR. Interleukin-1-beta induced corticosterone elevation and hypothalamic NE depletion is vagally mediated. *Brain Res Bull* 1995; 37: 605-10.
114. Gaykema RPA, Dijkstra I, Tilders FJH. Subdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. *Endocrinology* 1995; 136: 4717-20.
115. Sehic E, Blatteis CM. Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea-pigs. *Brain Res* 1996; 726: 160-6.
116. Goldbach JM, Roth J, Zeisberger E. Fever suppression by subdiaphragmatic vagotomy in guinea pigs depends on the route of pyrogen administration. *Am J Physiol (Regulatory Integrative Comp Physiol)* 1997; 41: R675-81.
117. Laye S, Bluthé RM, Kent S, et al. Subdiaphragmatic vagotomy blocks induction of IL-1-beta messenger-RNA in mice brain in response to peripheral LPS. *Am J Physiol (Regul Integr Comp Physiol)* 1995; 37: R1327-31.
118. Konsman JP, Luheshi GN, Bluthé RM, Dantzer R. The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. *Eur J Neurosci* 2000; 12: 4434-46.
119. Wiczorek M, Swiergiel AH, Pournajafi-Nazarloo H, Dunn AJ. Physiological and behavioural responses to interleukin-1, and LPS. *Physiol Behav* 2005; 85: 500-11.
120. Ross G, Roth J, Storr B, Voigt K, Zeisberger E. Afferent nerves are involved in the febrile response to injection of LPS into artificial subcutaneous chambers in guinea pigs. *Physiol Behav* 2000; 71: 305-13.
121. Romeo HE, Tio DL, Rahman SU, Chiappelli F, Taylor AN. The glossopharyngeal nerve as a novel pathway in immune-to-brain communication: relevance to neuroimmune surveillance of the oral cavity. *J Neuroimmunol* 2001; 115: 91-100.
122. Li S, Boackle SA, Holers VM, Lambiris JD, Blatteis CM. Complement component C5a is integral to the febrile response of mice to lipopolysaccharide. *Neuroimmunomod* 2005; 12: 67-80.
123. Perlik V, Li ZG, Goorha S, Ballou LR, Blatteis CM. LPS-activated complement, not LPS per se, triggers the early release of PGE₂ by Kupffer cells. *Am J Physiol (Regul Integr Comp Physiol)* 2005; 289: R332-9.
124. Morrison DC, Cochrane CG. Direct evidence for hageman-factor (factor-XII) activation by bacterial lipo-

- polysaccharides (endotoxins). *J Exp Med* 1974; 140: 797-811.
125. Herwald H, Morgelin M, Olsen A, et al. Activation of the contact-phase system on bacterial surfaces - a clue to serious complications in infectious diseases. *Nature Med* 1998; 4: 298-302.
126. Walker K, Dray A, Perkins M. Development of hyperthermia following intracerebroventricular administration of endotoxin in the rat: Effect of kinin B-1 and B-2 receptor antagonists. *Brit J Pharmacol* 1996; 117: 684-8.
127. Coelho MM, Oliveira CR, Pajolla GP, Calixto JB, Pela IR. Central involvement of kinin B-1 and B-2 receptors in the febrile response induced by endotoxin in rats. *Brit J Pharmacol* 1997; 121: 296-302.
128. Dray A, Bevan S. Inflammation and hyperalgesia: highlighting the team effort. *TIPS* 1993; 14: 287-90.
129. Tiffany CW, Burch RM. Bradykinin stimulates tumor necrosis factor and interleukin-1 release from macrophages. *FEBS Lett* 1989; 247: 189-92.
130. Vandekerckhove F, Opdenakker G, Vanranst M, et al. Bradykinin induces interleukin-6 and synergizes with interleukin-1. *Lymphokine Cytokine Res* 1991; 10: 285-9.
131. Ferreira SH, Lorenzetti BB, Poole S. Bradykinin initiates cytokine-mediated inflammatory hyperalgesia. *Brit J Pharmacol* 1993; 110: 1227-31.
132. Ferreira SH, Lorenzetti BB, Cunha FQ, Poole S. Bradykinin release of TNF- α plays a key role in the development of inflammatory hyperalgesia. *Agents Actions* 1993; 38 Suppl C:C7-9.
133. Cunha FQ, Lorenzetti BB, Poole S, Ferreira SH. The pivotal role of TNF α in the development of inflammatory hyperalgesia. *Brit J Pharmacol* 1992; 107: 660-4.
134. Dogan MD, Patel S, Rudaya AY, Steiner AA, Szekely M, Romanovsky AA. Lipopolysaccharide fever is initiated via a capsaicin-sensitive mechanism independent of the subtype-1 vanilloid receptor. *Brit J Pharmacol* 2004; 143: 1023-32.
135. Cunningham ET, Sawchenko PE. Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J Comp Neurol* 1988; 274: 60-76.
136. Plotsky PM, Cunningham ET, Widmaier EP. Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. *Endocr Rev* 1989; 10: 437-58.
137. Blatteis CM, Feleder C, Perlik V, Li SX. Possible sequence of pyrogenic afferent processing in the POA. *J Therm Biol* 2004; 29: 391-400.
138. Engblom D, Ek M, Saha S, Ericsson-Dahlstrand A, Jakobsson PJ, Blomqvist A. Prostaglandins as inflammatory messengers across the blood-brain barrier. *J Mol Med* 2002; 80: 5-15.
139. Bugajski J, Gadek-Michalska A, Bugajski A. Nitric oxide and prostaglandin systems in the stimulation of hypothalamic-pituitary-adrenal axis by neurotransmitters and neurohormones. *J Physiol Pharmacol* 2004; 55: 679-703.
140. Marz P, Cheng JG, Gadiant RA, et al. Sympathetic neurons can produce and respond to interleukin 6. *Proc Natl Acad Sci USA* 1998; 95: 3251-6.
141. De Jongh RF, Vissers KC, Meert TF, Booij LHDJ, De Deyne CS, Heylen RJ. The role of interleukin-6 in nociception and pain. *Anesth Analg* 2003; 96: 1096-103.
142. Breder CD, Smith WL, Raz A, et al. Distribution and characterization of cyclooxygenase immunoreactivity in the ovine brain. *J Comp Neurol* 1992; 322: 409-38.
143. Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons - regulation by synaptic activity and glucocorticoids. *Neuron* 1993; 11: 371-86.
144. Breder CD, Dewitt D, Kraig RP. Characterization of inducible cyclooxygenase in rat-brain. *J Comp Neurol* 1995; 355: 296-315.
145. Burian M, Geisslinger G. COX-dependent mechanisms involved in the antinociceptive action of NSAIDs at central and peripheral sites. *Pharmac Therapeut* 2005; 107: 139-54.
146. Vasquez E, Bar KJ, Ebersberger A, Klein B, Vanegas H, Schaible HG. Spinal prostaglandins are involved in the development but not the maintenance of inflammation-induced spinal hyperexcitability. *J Neurosci* 2001; 21: 9001-8.
147. Ranelis HJ, Griffin JD. The effects of prostaglandin E-2 on the firing rate activity of thermosensitive and temperature insensitive neurons in the ventromedial preoptic area of the rat hypothalamus. *Brain Res* 2003; 964: 42-50.
148. Hopkins SJ, Humphreys M. Simple, sensitive and specific bioassay of interleukin-1. *J Immunol Meth* 1989; 120: 271-6.
149. Hopkins SJ, Humphreys M. Bioassay of interleukin-1 in serum and plasma following removal of inhibitory activity with polyethylene glycol. *J Immunol Meth* 1990; 133: 127-31.

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