FEVER IN SYSTEMIC INFLAMMATION: ROLES OF PURINES

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1. ABSTRACT

Extracellular purine nucleotide and nucleoside signalling molecules, such as ATP and adenosine, acting through specific receptors (P2 and P1, respectively) play significant roles in the mechanisms underlying the febrile response. A variety of P2 and P1 receptor subunits have been identified in the hypothalamus, the area of the brain that orchestrates the febrile response. Importantly, both ATP and adenosine have been shown to modulate release and/or action of cytokines that are implicated in fever, as well as to be involved in the central mechanisms of cardiovascular and respiratory control. Our data indicate that at the level of the anterior hypothalamus extracellular ATP is involved in the control of the development of fever. A population of warm-sensitive neurones in the anterior hypothalamus is likely to be the site of action of ATP on body temperature. ATP-induced cytokine release does not appear to play a significant role in the hypothalamic mechanisms leading to the development of the febrile response. However, the blockade of fever by P2 receptor antagonists given systemically suggests that ATP-mediated signalling may play a role in the release of pyrogenic cytokines in the periphery. At the level of the anterior hypothalamus adenosine appears to be released tonically, and acts to maintain body temperature under afebrile conditions. There is also evidence that adenosine-mediated signalling may play a role in the hypothalamic mechanisms controlling the degree of body temperature increase during fever. Our investigations have identified possible mechanisms by which purines modulate the febrile response. The actions of purines on body temperature during fever are most likely "site specific" (brain vs. periphery), may or may not involve their effect on cytokine release and/or action, and are likely to involve P2 and P1 receptors of different subtypes. Further extensive studies are needed to elucidate these mechanisms in greater detail and may lead to the development of new approaches for modifying febrile, cytokine and acute-phase responses to infection.

2. INTRODUCTION

Systemic inflammation results in a number of behavioral and autonomic adaptive responses aimed at facilitating host resistance and slowing the growth of the pathogen. A regulated rise in body temperature, or fever, is one of these adaptive responses. For centuries fever served as an ultimate indicator of disease: in many cases the magnitude of the febrile response correlates well with severity of the inflammatory process. The mechanisms of fever, central and peripheral, have been under intense investigation over the last decades. As a result of these studies a variety of endogenous factors that mediate and/or modulate the febrile response have been identified. This review focuses on the roles played by extracellular purine signalling molecules adenosine 5'-triphosphate (ATP) and adenosine in the mechanisms of the febrile response during systemic inflammation.

There is strong evidence that extracellular ATP and adenosine, acting through specific ionotropic (P2X for ATP) and metabotropic (P2Y for ATP; and P1 for adenosine) receptors, have various functions in the brain as

well as in the peripheral tissues. Our interest in studying the role of ATP- and adenosine-mediated signalling in the mechanisms of the febrile response during systemic inflammation was triggered by the emerging evidence that both ATP and adenosine appear to be involved in regulating the production of cytokines. These include interleukin(IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α), which are essential for the development of fever. In addition, ATP- and adenosine-mediated signalling has been shown to play a role in the central mechanisms of cardiovascular and respiratory control. As development of fever involves changes in the pattern of peripheral blood flow and changes in respiration, we suggested that ATP and adenosine may play an additional role in the central mechanisms of the febrile response, possibly unrelated to the modulation of cytokine production. Although the roles of extracellular ATP and adenosine in central mechanisms of cardiovascular and respiratory control have been documented, the possibility that purinergic signalling may also be involved in central mechanisms of body temperature regulation has not been addressed until our recent studies.

Here, we review the literature that describes the roles played by ATP and adenosine in regulation of cytokine production, on the distribution of P2 and P1 receptors in the hypothalamus and the brainstem, as well as information on the roles played by ATP and adenosine in central mechanisms of cardiovascular and respiratory control. We integrate this literature with recent experimental data from our laboratories that indicates that ATP- and adenosine-mediated signalling may play an important role in the mechanisms controlling the febrile response.

3. ROLE OF EXTRACELLULAR ATP IN FEVER

There is growing evidence that extracellular ATP, known as an intracellular source of energy in metabolism, acting through ionotropic P2X and metabotropic P2Y receptors, acts as a fast signalling molecule in the brain and peripheral tissues (1, 2). In the central and peripheral nervous system ATP, acting via P2X receptors, is a fast excitatory neurotransmitter (1-3). P2X receptors are ATP-gated non-selective cation channels permeable to sodium, potassium and calcium ions (4-6). Seven subtypes of P2X receptors (P2X1-7) have been cloned and characterized in terms of agonist/antagonist selectivity (3-6). In addition, eight subtypes of G protein-coupled P2Y receptors for ATP have been described (7).

Extracellular ATP working through these specific cell-surface receptors has numerous physiological functions. However, when its role in fever is considered two aspects seem to be potentially important: (i) the ability to induce a release of cytokines; and (ii) an involvement in central mechanisms of cardiovascular and respiratory control. In addition, several types of the P2 receptor subunits have been identified in the hypothalamus, the area of the brain responsible for the orchestration of the febrile response.

3.1. Effect of ATP on cytokine production

Significant evidence indicates that many circulating cytokines, such as IL-1β, IL-6, TNFα and others, act as endogenous pyrogens and are responsible for the induction and maintenance of fever by raising the "set point" for body temperature regulation (8, 9). Based on the extensive evidence indicating crucial roles for IL-1β, IL-6, TNFα and other cytokines in the development of the febrile response, we have suggested earlier (10) that any endogenous factor, such as, for example, ATP or adenosine, involved in the mechanisms of cytokine production or clearance may also play an important role in fever.

The functional role of ATP-mediated purinergic signalling in immune and inflammatory responses has been under intense investigation in recent years (11, 12), and particular attention being paid to interactions between extracellular ATP and cytokines. ATP has been shown to induce the release of IL-1β (13-16), IL-6 (17), IL-8 (18), IL-18 (14) and TNFα (19) from various cells, including microglial cells. There is convincing evidence that ATP induces cytokine release by acting at ionotropic $P2X_7$ receptors (14, 18-21). In mice lacking $P2X_7$ receptor, Labasi et al. (22) demonstrated an attenuation of the systemic inflammatory response. Interestingly, Denlinger et al. (23) showed that the $P2X_7$ C-terminal domain contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide (LPS). Thus, it appears that the ionotropic $P2X_7$ receptors expressed by immune cells, and therefore ATP-induced responses mediated by this receptor, can be directly influenced by LPS binding. ATP at these very same receptors induces release of various cytokines, implicated in fever.

These data showing that ATP working through $P2X_7$ receptors induces cytokine release, suggest that purinergic signalling may play an essential role in the development of the febrile response. However, most of the studies mentioned above describe the effect of ATP on cytokine production by various immune cells. Thus, the functional role of extracellular ATP-mediated purinergic signalling in "in vivo" cytokine release as yet remains unknown.

3.2. P2 receptors in the hypothalamus

P2 receptors are expressed throughout the hypothalamus. Using immunohistochemistry Xiang et al. (24) have demonstrated that in the rat hypothalamus $P2X_2$ receptor subtype expressing neurones are present in the preoptic and retrochiasmatic areas, paraventricular, supraoptic, periventricular, arcuate, circular, and ventral tuberomammillary and other nuclei. These data were confirmed by Kanjhan et al. (25) using immunohistochemistry with another antibody as well as in situ hybridization. Studies involving our laboratory also give similar results (26, 27). Loesch and Burnstock (28) have demonstrated the expression of $P2X_6$ receptor subtype in the rat hypothalamus, while Shibuya et al. (29) have shown that mRNAs for $P2X_2$, $P2X_3$, $P2X_4$, $P2X_6$ and $P2X_7$ receptors are expressed in the supraoptic nucleus. Furthermore, Whitlock et al. (30) found that $P2X_3$, $P2X_4$ and $P2X_6$ subunits are likely to be expressed in parvocellular component of the rat hypothalamic paraventricular nucleus, in addition to $P2X_2$. Recently,

Kittner*et al.* (31) have demonstrated that metabotropic P2Y$_1$ receptors are also present in the rat hypothalamus.

Thus, there is significant evidence indicating that ionotropic P2X and metabotropic P2Y receptors are expressed in the hypothalamus – the area of the brain responsible for fine regulation of body temperature and the development of fever. However, any functional role of ATP-mediated purinergic signalling in the mechanisms of thermoregulation during fever had not received attention until our recent studies (32-34).

3.3. Purinergic signalling in central mechanisms of cardiovascular and respiratory control

In recent years the evidence has grown that extracellular ATP, acting at P2X receptors, is involved in the central nervous mechanisms of cardiovascular and respiratory control. There is evidence that ATP, acting on P2 receptors in the nucleus tractus solitarii (NTS) of the medulla oblongata is involved in baroreflex regulation of heart rate (35, 36). Recent studies involving our laboratory demonstrated that NTS P2 purinergic receptors also play a role in the neurotransmission of the parasympathetic component of the chemoreceptor reflex (37). In addition, ATP, acting on P2X receptors, within the ventrolateral medulla has been shown to play a role in regulation of vasomotor tone and sympathetic activity (38, 39), and that P2X receptors in this area of the brainstem mediate hypercapnia-induced changes in respiration (40).

The role for purinergic signalling in central cardiovascular and respiratory control is strongly supported by the results of immunohistochemical and *in situ* hybridization studies indicating that P2X receptors of different types are abundant in the brainstem (25-27, 41-45).

Taken together these data indicate that at least at the brainstem level ATP-mediated purinergic signalling plays an important role in central mechanisms of cardiovascular and respiratory regulation. As thermoregulatory, cardiovascular and respiratory systems are intimately linked, and regulation of body heat exchange to the environment includes changes in the pattern of peripheral blood flow and changes in breathing, there are grounds to suggest that ATP, acting through its specific receptors in the hypothalamus and/or brainstem exert a profound influence on thermoregulation.

3.4. Hypothalamic extracellular ATP and its role in regulation of body temperature during fever

In our pharmacological study (32-34), experiments were designed to determine whether ATP was acting on hypothalamic P2 receptors that were involved in regulation of body temperature in febrile and afebrile rats. The effects of the stable ATP analogue α,β-methyleneATP (α,β-meATP) and P2 receptor antagonists suramin and pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) when injected into the anterior hypothalamus or into third cerebral ventricle (i.c.v.) were tested on body temperature in conscious rats during fever induced by bacterial LPS.

Activation of brain P2 receptors by injection of α,β-meATP did not affect body temperature when compared to pre-injection levels in afebrile rats maintained at an ambient temperature of 25°C. However, α,β-meATP when injected i.c.v. or directly into the anterior hypothalamus caused a profound fall in body temperature during LPS-induced fever in rats (Figure 1). The P2 receptor antagonist suramin attenuated significantly this effect of α,β-meATP, indicating that it is mediated via as yet undefined P2 receptors.

In afebrile rats the pharmacological blockade of central P2 receptors with suramin resulted in a marked and long-lasting increase in body temperature (Figure 2A), similar in magnitude and time-course to the febrile response induced by LPS. An increase in body temperature of similar amplitude was also observed in rats treated with PPADS (Figure 2B). Fever was initiated more rapidly in rats treated i.c.v. with suramin or PPADS, but its late phase was unaffected (Figures 3A and 3B). Potentiation of the initial phase of fever when P2 receptor antagonists were given i.c.v. before LPS injection was rather minor. Furthermore, changes in the body temperature of rats treated with suramin or PPADS followed by LPS did not exceed changes observed in rats treated with either of these antagonists alone. In rats that were already febrile, neither suramin nor PPADS altered body temperature (Figure 3C), indicating that development of the febrile response prevents the effects of P2 receptor antagonists on thermoregulation. Thus, it appears that the magnitude of the changes in body temperature evoked by activation and blockade of central P2 receptors during fever significantly differs from that observed in afebrile rats at the same ambient temperature. Accordingly we suggested that hypothalamic warm-sensitive neurones are the likely targets for the action of ATP revealed by the use of the ATP analogue and P2 receptor antagonists in our experiments (32).

This hypothesis is supported by the results of our recent *in vitro* studies (34). Here we have been found that the bath application of ATP increases the firing rate of 60% of warm-sensitive neurones in brain slices containing the preoptic area/anterior hypothalamus (Figure 4). It is well-known that about 30% of the anterior hypothalamic neurones are warm-sensitive, and these neurones are believed to be the site of action of pyrogens, which cause fever by reducing their discharge (46). Thus, we suggest that hypothalamic warm-sensitive neurones are under a tonic P2X receptor-mediated ATP-induced excitation. This would imply that in conditions close to thermoneutrality, P2 receptor blockade would be expected to result in a marked increase in body temperature as a consequence of a decrease in the activity of these neurones. In febrile rats P2 receptor blockade is unable to induce an increase in body temperature, since the activity of these

hypothalamic neurones is already reduced due to a pyrogen-evoked inhibition. In addition the activation of P2 receptors by application of exogenous agonist (α,β-meATP) will increase the activity of these same hypothalamic warm-sensitive neurones, resulting in a marked and immediate drop in body temperature, not seen
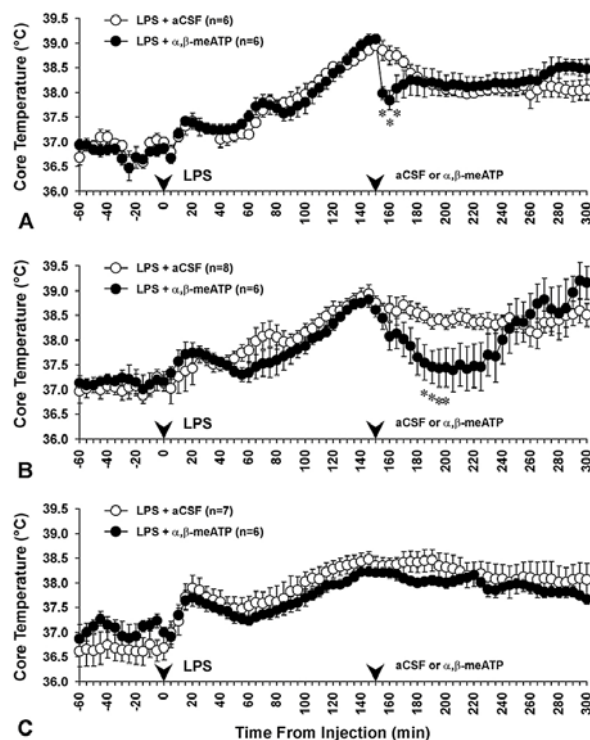
Figure 1. Effect of intracerebroventricular [A], intrahypothalamic [B] and intraperitoneal [C] injection of the ATP analogue α,β-methyleneATP (α,β-meATP; 0.2 µmol) or artificial cerebrospinal fluid (aCSF) on body temperature at the peak of fever induced by *E.coli* lipopolysaccharide (LPS, 50 µg kg^{-1}) in rats (25°C ambient temperature). Data are presented as mean ± SE. Numbers in parentheses indicate sample sizes. α,β-meATP or aCSF were injected 2.5 h after intraperitoneal injection of LPS. Arrowheads indicate time of injections. *Significant difference in body temperature between LPS-treated rats injected into the third cerebral ventricle [A] or into the anterior hypothalamus [B] with α,β-meATP and rats injected with aCSF, $p<0.05$. (Data are from Gourine *et al.* [32]).
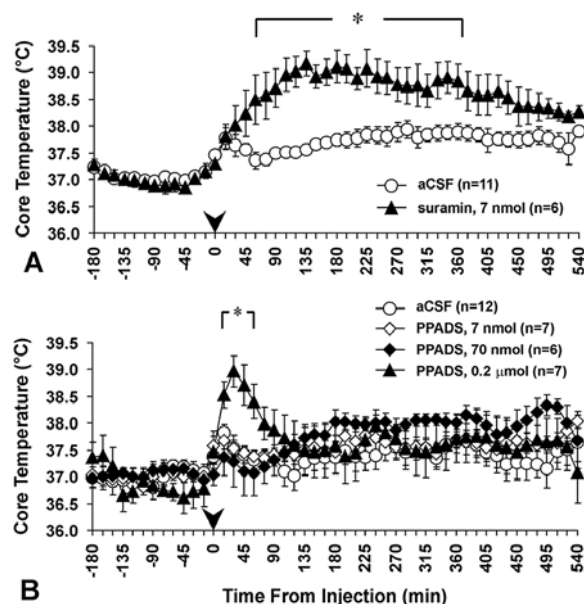

Figure 2. Effect of intracerebroventricular injection of the P2 receptor antagonists suramin (7 nmol [A]) and pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 7 nmol, 70 nmol and 0.2 µmol [B]) or artificial cerebrospinal fluid (aCSF) on body temperature in rats at 25°C ambient temperature. Data are presented as mean ± SE. Numbers in parentheses indicate sample sizes. Arrowhead indicates time of injections. *Significant difference in body temperature between rats treated into the third cerebral ventricle with suramin [A] or PPADS [B] and rats treated with aCSF, $p<0.05$. (Data are from Gourine *et al.* [32]).
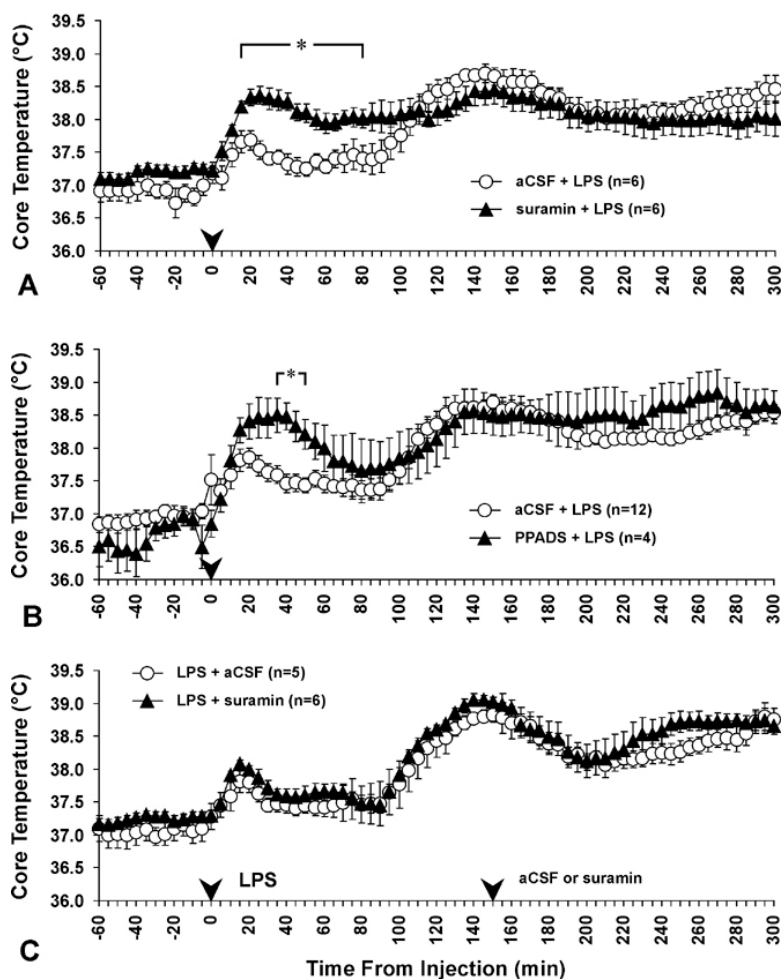
Figure 3. Effect of intracerebroventricular injection of the P2 receptor antagonists suramin (7 nmol [A]) and pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 70 nmol [B]) or artificial cerebrospinal fluid (aCSF) on body temperature during fever induced by *E.coli* lipopolysaccharide (LPS, 50 µg kg^{-1}) in rats (25°C ambient temperature). Suramin was injected immediately before [A] or 2.5 h after [C] intraperitoneal injection of LPS. PPADS was injected immediately before [B] intraperitoneal injection of LPS. Data are presented as mean ± SE. Numbers in parentheses indicate sample sizes. Arrowhead indicates time of injections. *Significant difference in body temperature between LPS-treated rats injected into the third cerebral ventricle with suramin [A] or PPADS [B] and rats injected with aCSF, $p<0.05$. (Data are from Gourine *et al.* [32]).
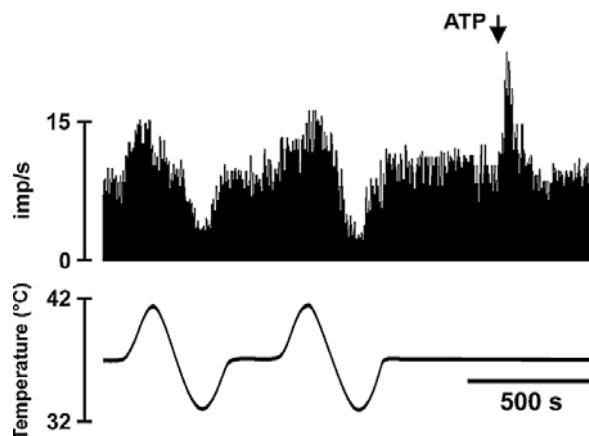

Figure 4. Effect of ATP (50 mM) on the firing rate of the warm-sensitive neurone in the anterior hypothalamus *in vitro* (slices 400 µM). Lower panel shows temperature changes in the incubation chamber (*Gourine, Melenchuk, Poputnikov, Gourine, & Spyer, unpublished observations*).

in afebrile animals. At present, the identity of P2 receptors that are expressed by these hypothalamic warm-sensitive neurones remains unknown.

Since fever is initiated by IL-1β (and probably other cytokines) at the level of the anterior hypothalamus (47, 48) and ATP is a potent inducer of cytokine release, including IL-1β (15, 16, 19, 21), we proposed initially that ATP might act to induce the release of cytokines to evoke fever. ATP induces cytokine release by acting at ionotropic $P2X_7$ receptors (see above), which are present in the anterior hypothalamus and sensitive to the antagonist PPADS (49). If the above hypothesis is correct, and ATP, via cytokine release in the anterior hypothalamus, plays a role in the febrile response, an attenuation of fever by PPADS would have been expected. However, fever was virtually unaffected, or even enhanced, following administration of PPADS, indicating that at the hypothalamic level either ATP is not involved in the mechanisms of cytokine release, or, alternatively, that ATP-induced cytokine production is not essential for the development of LPS-induced fever.

From these data we conclude that extracellular ATP within the anterior hypothalamus, acting through as yet undefined P2 receptors, is involved in the mechanisms of fever, limiting the magnitude of the febrile response. A population of warm-sensitive neurones in the anterior hypothalamus expressing these receptors is likely to be the site of action of ATP on body temperature. It appears that the ability of ATP to induce cytokine release does not play significant role in the hypothalamic mechanisms leading to the development of the febrile response.

3.5. "Peripheral" vs "central" ATP-mediated signalling in the mechanisms of the febrile response

Recent observations from studies in our laboratories indicate that in the periphery ATP-mediated purinergic signalling may play an additional and, apparently very significant, role in the mechanisms leading to the development of the febrile response.

When the P2 receptor antagonists suramin and PPADS were injected intraperitoneally (i.p.) at the same doses that were shown to influence body temperature after i.c.v. injection, they failed to induce any changes in body temperature in either afebrile or febrile rats (32). However, when these antagonists were given i.p. in amounts sufficient to affect the function of peripheral P2 receptors, a dose-dependent attenuation of the fever induced by LPS was observed (*Gourine, Poputnikov, Gourine & Spyer, unpublished observations*). The febrile response was blocked completely by suramin (100 mg/kg) and attenuated significantly by PPADS (25 mg/kg). Both P2 receptor antagonists had no effect on body temperature in afebrile animals.

Thus, if ATP acting via $P2X_7$ receptors is indeed involved in *in vivo* cytokine production following an LPS challenge, we propose that the attenuation of fever that is induced by systemic treatment with P2 receptor antagonists may result from an inhibition and/or an alteration in LPS-induced cytokine release. Experiments designed to test this possibility are currently in progress.

3.6. Conclusion: Role of extracellular ATP in fever

Our recent results indicate that in the anterior hypothalamus extracellular ATP is involved in the regulation of body temperature during fever. A population of warm-sensitive neurones in the anterior hypothalamus is one of probably several central sites of ATP action on body temperature regulation. ATP also has several important functions at the level of the medulla oblongata and contributes to the control of cardiovascular and respiratory function. These actions of ATP in the medulla could be equally important in regulation of body temperature during fever, however, their contribution as yet remains unknown. ATP-induced cytokine release in the brain does not appear to play a significant role in the mechanisms leading to the development of the febrile response. However, the blockade of fever by P2 receptor antagonists given systemically suggests that ATP-mediated signalling may play a role in the release of pyrogenic cytokines in the periphery.

4. ROLE OF ADENOSINE IN FEVER

Adenosine is considered to be a major non-peptide neuromodulator in the brain and its release increases dramatically in conditions of metabolic stress, such as systemic hypoxia, brain ischemia, etc. (for reviews, see [50, 51]). Adenosine may be produced intracellularly from degradation of AMP, and then be released from the cell via nucleoside transporters, or extracellularly from the breakdown of ATP by 5'-nucleotidases (52, 53). Adenosine exerts its effects via activation of cell surface G-protein-coupled adenosine receptors (7). Four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, A_3) have been cloned and characterized (7).

Adenosine acting through these different receptor subtypes has a variety of functions within the central nervous system and in the peripheral tissues. However, as with ATP, when its role in the febrile response is considered two aspects of its action are important: (i) its ability to modulate the production and action of cytokines; and (ii) an involvement in central mechanisms of cardiovascular and respiratory control. In addition, adenosine receptors have been found in the hypothalamus, suggesting that adenosine might modulate the activity of hypothalamic neurones.

4.1. Effect of adenosine on the production and action of cytokines

There is a vast amount of literature, impossible to list here, in which adenosine has been implicated in various aspects of the immune response. The general consensus is that, in contrast to ATP, adenosine action is predominantly anti-inflammatory. For the purpose of the current paper we review and discuss only data pertinent to the action of adenosine on the production and action of cytokines, involved in the development of the febrile response.

Adenosine has been shown to inhibit the production of TNFα and IL-12 in macrophages and monocytes (54-56). Adanin *et al.* (57) demonstrated that the adenosine deaminase inhibitor pentostatin attenuates the elevation in serum TNF-α and IL-1β during endotoxemia and sepsis, while the adenosine receptor antagonist 8-sulfophenyltheophylline had the opposite effect. This effect of adenosine on TNFα expression is thought to be mediated predominantly by A_3 receptors (55, 56), however A_1 and A_{2a} receptors may also be involved (54, 58). Interestingly, the adenosine metabolite inosine is also able to inhibit the production of several proinflammatory cytokines, including IL-1β, TNFα, IL-12 and others (59). IL-6 has been found to be strongly inhibited by A_1 receptor activation in splenic tissue (60). Furthermore, adenosine enhances IL-10 secretion (58), but suppresses TNFα-induced activation of nuclear factor – kappaB (61). These data indicate that the release (and perhaps action) of pro-inflammatory (pyrogenic) cytokines is suppressed, while the release of anti-inflammatory (antipyretic) cytokines is enhanced by adenosine. Interestingly, there is evidence that bacterial endotoxin LPS can bind and activate A_1 adenosine receptors on endothelial cells (62), indicating that a direct action of LPS on adenosine receptors may modulate adenosine responses evoked at these receptors.

Thus, there is convincing evidence that adenosine working through its specific cell-surface receptors modulates the production and action of cytokines involved in the development and maintenance of the febrile response, including IL-1β, TNFα, IL-6, and others. These data show that adenosine modulates cytokine production and action in *in vitro* systems, but also points to a potentially significant "*in vivo*" role of adenosine-mediated signalling in systemic inflammation, cytokine production and ultimately, in the mechanisms of the febrile response.

4.2. Adenosine receptors in the hypothalamus and brainstem. Adenosine in central mechanisms of cardiovascular and respiratory control.

It is now widely accepted that central neurones can directly release significant amounts of adenosine, in quantities sufficient to activate adenosine receptors and to modulate neuronal activity (for review see [50]). A "pool" of adenosine formed following breakdown of extracellular ATP with the help of 5'-nucleotidase activities is equally important in modulation of activity of neurones that express adenosine receptors.

Adenosine receptors have been demonstrated to be widespread in the central nervous system (63). A_1 and A_{2A} receptor subtypes are found in the hypothalamus (64-66).The lower brainstem has a very high uptake rate for adenosine (67) and a high density of adenosine deaminase (68) and adenosine receptors (63, 69, 70).

A close relationship exists between adenosine production and the modulation of cardiorespiratory function within the medulla oblongata. Adenosine has been shown to modulate the ongoing activity of neurones in the rostral ventrolateral medulla by acting at both A_1 and A_{2a} receptors (71). The application of adenosine to the NTS evokes profound changes in cardiovascular and respiratory activity (35, 72). A tonic release of adenosine is important for central respiratory control as adenosine itself and adenosine receptor agonists depress, and adenosine receptor antagonists enhance respiratory activity *in vivo* and *in vitro* (73-75).

In an extensive series of experiments, adenosine has been shown to act within the NTS to modulate the baroreceptor and chemoreceptor reflexes, probably via an action on the release of other neurotransmitters in this area (70, 76; see [77] for a review). Adenosine appears also to play an important role in the manifestation of some components of the defense reaction evoked by electrical stimulation of the hypothalamus (78). In more recent studies we have demonstrated that adenosine is released in the NTS at the right time and in sufficient quantities to contribute to the cardiovascular components of the defense reaction (79).

These data indicate that adenosine receptors are abundant in the hypothalamus and the brainstem. At the level of the brainstem adenosine appears to play an important role in cardiovascular and respiratory control. The regulation of body heat exchange with the environment depends upon changes in both cardiovascular and respiratory systems. Accordingly, it might be expected that adenosine-mediated signalling would play some role in the central mechanisms of thermoregulation in control conditions and during fever. When the role of central adenosine in fever is considered the presence of adenosine receptors in the NTS and action of adenosine on these receptors are of a particular interest. First, the NTS is the site of termination of vagal afferents, some of which have been proposed to be involved in the initiation of fever by "transmitting pyrogenic signal" from the periphery to the brain (80-82). Thus, adenosine could modulate transmission of this "signal" at the NTS level. Second, the NTS is located in close proximity to the floor of the fourth ventricle and therefore, can be easily reached by the substances injected into the lateral or third cerebral ventricles. Therefore, changes in body temperature evoked by adenosine receptor agonists and antagonists injected i.c.v. (reviewed and discussed below) may be mediated partially by activation or blockade of NTS adenosine receptors.

4.3 Adenosine and its role in regulation of body temperature during fever.

More than a decade ago Ticho & Radulovacki (83) demonstrated that the nonselective adenosine A_1/A_2 receptor agonist N-ethyl-carboxamido-adenosine as well as the adenosine A_1 receptor agonist cyclopentyladenosine can induce drop in body temperature in rats after bilateral microinjections into the preoptic area of the hypothalamus. A decrease in body temperature also followed systemic administration of adenosine, or adenosine receptor agonists (84). Anderson *et al.* (85) investigated the effects of a range of adenosine receptor-selective ligands on body temperature following i.c.v. and intraperitoneal injection in conscious mice and concluded that the drop in body temperature induced by adenosine analogues in these
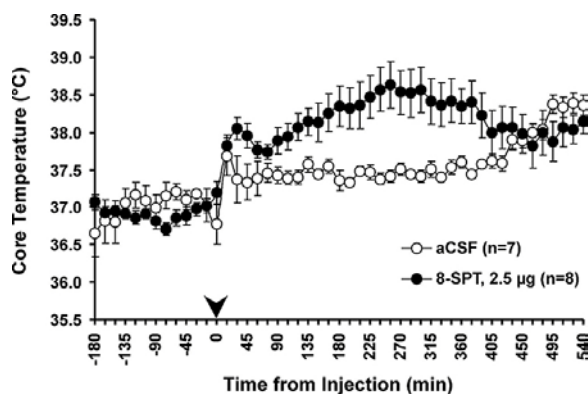
Figure 5. Effect of intrahypothalamic injection of the adenosine receptor antagonist 8-(p-sulfophenyl)-theophylline (8-SPT) or artificial cerebrospinal fluid (aCSF) on body temperature in rats. Data are presented as mean ± SE. Numbers in parentheses indicate sample sizes. Arrowhead indicate time of injections. (*Gourine, Poputnikov, Gourine, & Spyer, unpublished observations*).
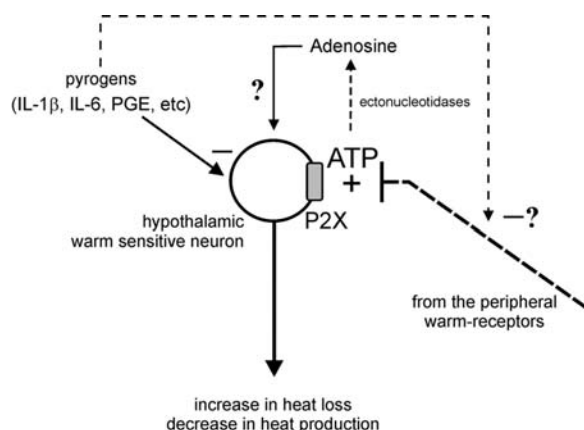

Figure 6. Hypothetical model of ATP involvement in the central mechanisms of thermoregulation. We suggest that ATP acting via, as yet unknown, P2X receptors is the mediator in the neuronal pathway from the peripheral warm receptors to the hypothalamic warm sensitive neurons (discussed in detail by Gourine *et al.* [32]). Blockade of these receptors in the hypothalamus of afebrile rats results in an increase in body temperature. During fever activation of these receptors by α,β-meATP results in an immediate decrease in body temperature, not seen in afebrile animals. Blockade of central P2X receptors at the time when fever already developed does not alter febrile body temperature, suggesting that development of the febrile response results in inhibition of the ATP transmission mediated by these receptors. The influence of adenosine on hypothalamic warm sensitive neurons still remains unknown. IL-1β – interleukin-1β, PGE – prostaglandins G.

animals is mediated via adenosine A_1 receptors located within the central nervous system. Matuszek and Gagalo (86) reported an attenuation of fever and inhibition of metabolic rate in rabbits after intravenous administration of

A_1 (N6-cyclohexyladenosine) and A_2 (5'-N-ethylcarboxamidoadenosine) receptor agonists. However, these studies only described pharmacological effects of adenosine receptor agonists on body temperature, and the physiological role of adenosine-mediated signalling in thermoregulation remained uncertain. Recently, Branco *et al.* (87) demonstrated that adenosine mediates hypoxia-induced decrease in body temperature in toads. Our latest studies suggest that adenosine acting at the level of the anterior hypothalamus may play an important role in regulation of body temperature in mammals as well.

We have found that in conscious rats blockade of adenosine receptors in the brain by intracerebroventricular injection of 8-(p-sulfophenyl)-theophylline (8-SPT) results in a marked and long-lasting increase in body temperature, similar in magnitude and time-course to the febrile response induced by LPS (*Gourine, Poputnikov, Gourine & Spyer, unpublished observations*). An increase in body temperature was also observed following unilateral microinjection of 8-SPT directly into the anterior hypothalamus (Figure 5). The febrile responses evoked by bacterial LPS (50 µg/kg, i.p.) were markedly exaggerated in rats pretreated intracerebroventricularly with the adenosine receptor antagonist 8-SPT (*Gourine, Poputnikov, Gourine & Spyer, unpublished observations*).

4.4. Conclusion: Role of adenosine in fever

From these limited available data we suggest that adenosine is released tonically in the anterior hypothalamus and that this has a tonic action in the maintenance of body temperature under euthermic conditions. Circumstantial evidence also suggests that adenosine-mediated signalling may as well play a role in the hypothalamic mechanisms controlling the magnitude of the febrile response. The actions of the "adenosine pool" involved in the control of cardiovascular and respiratory activities at the level of the medulla oblongata in the mechanisms of fever could be equally important, however, their contribution remains unknown. Thus, significantly more studies are needed to reveal the precise role of brain adenosine in fever, its site(s) of action (hypothalamus *vs.* brainstem), as well as the nature of the receptors that mediate the effect of adenosine on body temperature in febrile conditions. The importance of interactions between adenosine and cytokines (IL-1β, IL-6, TNFα, etc) both in the brain and in the periphery, in relation to regulation of body temperature during fever, still have to be demonstrated.

5. PERSPECTIVES

Considerable published material and the results of our recent studies indicate that extracellular purines, ATP and adenosine, play significant roles in fever. The actions of purines on body temperature during fever are most likely "site specific" (brain *vs.* periphery), may or may not involve their effect on cytokine release and/or action, and may be mediated by P2 and P1 receptors of different subtypes. Our investigations have indicated only some of the possible mechanisms by which purines modulate the febrile response (Figures 6 and 7). Extensive additional
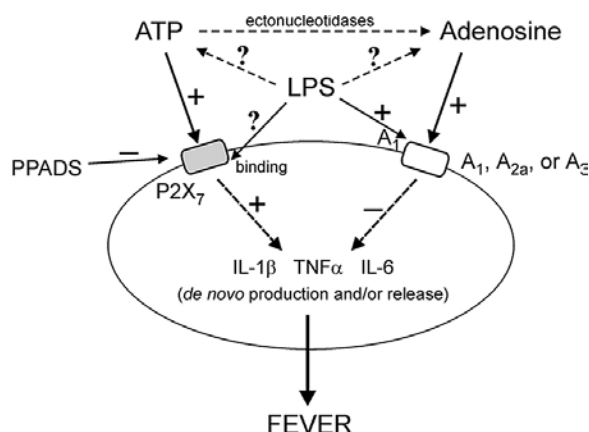
Figure 7. Schematic model of ATP and adenosine involvement in modulation of production and/or release of cytokines, implicated in the development of fever. ATP induces interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) release via activation of $P2X_7$ receptors in various immune cell types. Adenosine inhibits release of these cytokines via actions at A_1, A_{2a}, or A_3 receptors. Bacterial endotoxin lipopolysccharide (LPS) may bind $P2X_7$ receptors, which contain a conserved LPS-binding domain, as well as interact with adenosine A1 receptors. As yet, it remains unknown whether LPS induces changes in tissue and plasma levels of ATP and adenosine. Systemic treatment with P2 receptor antagonist PPADS results in a significant attenuation of fever. However, the role of ATP-mediated signaling via P2X7 receptors in the in vivo cytokine production and fever remains to be investigated in detail.

studies are needed to elucidate fully these mechanisms. In this respect we consider that it is imperative to determine:

1. in real time whether the extracellular concentration of ATP and adenosine in the brain and periphery (i.e. in the blood) increases during the development of fever induced by systemic inflammation;

2. the effects of intrahypothalamic microinjections and systemic administrations of selective agonists and antagonists of different subtypes of P2X, P2Y and adenosine receptors on body temperature during fever induced by systemic inflammation;

3. the effects of P2 and adenosine receptor activation and blockade on cytokines (IL-1β, IL-6, TNFα, etc) expression in the brain and in the periphery during fever induced by systemic inflammation;

4. the profile of P2 and P1 receptor proteins contained within hypothalamic thermosensitive neurones.

These studies of the possible mechanisms of purinergic signalling involvement in fever may lead to the development of new approaches to modulate the febrile, cytokine and acute-phase responses to infection.

6. ACKNOWLEDGEMENT

7. REFERENCES

1. Edwards FA, Gibb AJ, Colquhoun D: ATP receptor-mediated synaptic currents in the central nervous system. *Nature* 359, 144-147 (1992)

2. Burnstock G: Current status of purinergic signalling in the nervous system. *Prog Brain Res* 120, 3-10 (1999)

3. Buell G, Collo G, Rassendren F: P2X receptors: an emerging channel family. *Eur J Neurosci* 8, 2221-2228 (1996)

4. North RA: Molecular physiology of P2X receptors. *Physiol Rev* 82, 1013-1067 (2002)

5. North RA, Surprenant A: Pharmacology of cloned P2X receptors. *Annu Rev Pharmacol Toxicol* 40, 563-580 (2000)

6. Ralevic V, Burnstock G: Receptors for purines and pyrimidines. *Pharmacol Rev* 50, 413-492 (1998)

7. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J: International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 53, 527-552 (2001)

8. Kluger MJ: Fever: role of pyrogens and cryogens. *Physiol Rev* 71, 93-127 (1991)

9. Kluger MJ, Kozak W, Leon LR, Soszynski D, Conn CA: Cytokines and fever. *Neuroimmunomodulation* 2, 216-223 (1995)

10. Gourine AV, Gourine VN, Tesfaigzi Y, Caluwaerts N, Van Leuven F, Kluger MJ: Role of α2-macroglobulin in fever and cytokine responses induced by lipopolysaccharide in mice. *Am J Physiol Regul Integr Comp Physiol* 283, R218-R226 (2002)

11. Dubyak GR: Purinergic signaling at immunological synapses. *J Auton Nerv Syst* 81, 64-68 (2000)

12. la Sala A, Ferrari D, Di Virgilio F, Idzko M, Norgauer J, Girolomoni G: Alerting and tuning the immune response by extracellular nucleotides. *J Leukoc Biol* 73, 339-343 (2003)

13. Chakfe Y, Seguin R, Antel JP, Morissette C, Malo D, Henderson D, Seguela P: ADP and AMP induce interleukin-1β release from microglial cells through activation of ATP-primed $P2X_7$ receptor channels. *J Neurosci* 22, 3061-3069 (2002)

14. Mehta VB, Hart J, Wewers MD: ATP-stimulated release of interleukin (IL)-1β and IL-18 requires priming by lipopolysaccharide and is independent of caspase-1 cleavage. *J Biol Chem* 276, 3820-3826 (2001)

15 Perregaux DG, Gabel CA: Post-translational processing of murine IL-1: evidence that ATP-induced release of IL-1α and IL-1β occurs via a similar mechanism. *J Immunol* 160, 2469-2477 (1998)

16. Perregaux DG, McNiff P, Laliberte R, Conklyn M, Gabel CA: ATP acts as an agonist to promote stimulus-induced secretion of IL-1β and IL-18 in human blood. *J Immunol* 165, 4615-4623 (2000)

17. Inoue K: Microglial activation by purines and pyrimidines. *Glia* 40, 156-163 (2002)

18. Idzko M, Panther E, Bremer HC, Sorichter S, Luttmann W, Virchow CJ Jr, Di Virgilio F, Herouy Y, Norgauer J, Ferrari D: Stimulation of P2 purinergic receptors induces the

release of eosinophil cationic protein and interleukin-8 from human eosinophils. *Br J Pharmacol* 138, 1244-1250 (2003)

19. Hide I, Tanaka M, Inoue A, Nakajima K, Kohsaka S, Inoue K, Nakata Y: Extracellular ATP triggers tumor necrosis factor-α release from rat microglia. *J Neurochem* 75, 965-972 (2000)

20. Baraldi PG, del Carmen Nunez M, Morelli A, Falzoni S, Di Virgilio F, Romagnoli R: Synthesis and biological activity of N-arylpiperazine-modified analogues of KN-62, a potent antagonist of the purinergic $P2X_7$ receptor. *J Med Chem* 46, 1318-1329 (2003)

21. Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH, Griffiths RJ, Gabel CA: Altered cytokine production in mice lacking $P2X_7$ receptors. *J Biol Chem* 276, 125-132 (2001)

22. Labasi JM, Petrushova N, Donovan C, McCurdy S, Lira P, Payette MM, Brissette W, Wicks JR, Audoly L, Gabel CA: Absence of the $P2X_7$ receptor alters leukocyte function and attenuates an inflammatory response. *J Immunol* 168, 6436-6445 (2002)

23. Denlinger LC, Fisette PL, Sommer JA, Watters JJ, Prabhu U, Dubyak GR, Proctor RA, Bertics PJ: Cutting edge: the nucleotide receptor $P2X_7$ contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J Immunol* 167, 1871-1876 (2001)

24. Xiang Z, Bo X, Oglesby I, Ford A, Burnstock G: Localization of ATP-gated $P2X_2$ receptor immunoreactivity in the rat hypothalamus. *Brain Res* 813, 390-397 (1998)

25. Kanjhan R, Housley GD, Burton LD, Christie DL, Kippenberger A, Thorne PR, Luo L, Ryan AF: Distribution of the $P2X_2$ receptor subunit of the ATP-gated ion channels in the rat central nervous system. *J Comp Neurol* 407, 11-32 (1999)

26. Gourine AV, Yao ST, Lawrence AJ, Spyer KM: Colocalization of nitric oxide synthase and $P2X_2$ receptor immunoreactivities in neurones of the rat hypothalamus and medulla. *J Physiol* 539, 109P (2002)

27. Yao ST, Gourine AV, Spyer KM, Barden JA, Lawrence AJ: Localisation of $P2X_2$ receptor subunit immunoreactivity on nitric oxide synthase expressing neurones in the brain stem and hypothalamus of the rat: a fluorescence immunohistochemical study. *Neuroscience* 121, 411-419 (2003)

28. Loesch A, Burnstock G: Immunoreactivity to $P2X_6$ receptors in the rat hypothalamo-neurohypophysial system: an ultrastructural study with extravidin and colloidal gold-silver labelling. *Neuroscience* 106, 621-631 (2001)

29. Shibuya I, Tanaka K, Hattori Y, Uezono Y, Harayama N, Noguchi J, Ueta Y, Izumi F, Yamashita H: Evidence that multiple P2X purinoceptors are functionally expressed in rat supraoptic neurones. *J Physiol* 514, 351-367 (1999)

30. Whitlock A, Burnstock G, Gibb AJ: The single-channel properties of purinergic P2X ATP receptors in outside-out patches from rat hypothalamic paraventricular parvocells. *Pflugers Arch* 443, 115-122 (2001)

31. Kittner H, Franke H, Fischer W, Schultheis N, Krugel U, Illes P: Stimulation of $P2Y_1$ receptors causes anxiolytic-like effects in the rat elevated plus-maze: implications for the involvement of $P2Y_1$ receptor-mediated nitric oxide production. *Neuropsychopharmacology* 28: 435-444 (2003)

32. Gourine AV, Melenchuk EV, Poputnikov DM, Gourine VN, Spyer KM: Involvement of purinergic signalling in central mechanisms of body temperature regulation in rats. *Br J Pharmacol* 135, 2047-2055 (2002)

33. Gourine VN, Melenchuk EV, Poputnikov DM, Gourine AV, Spyer KM: Evidence for involvement of ATP in regulation of body temperature during fever in rats. *J Physiol* 533, 91P (2001)

34. Gourine VN, Melenchuk EV, Poputnikov DM, Gourine AV, Spyer KM: Central nervous system site of ATP action on body temperature during fever in rats. *J Physiol* 547P, (2003)

35. Phillis JW, Scislo TJ, O'leary DS: Purines and the nucleus tractus solitarius: effects on cardiovascular and respiratory function. *Clin Exp Pharmacol Physiol* 24, 738-742 (1997)

36. Scislo TJ, Ergene E, O'Leary DS: Impaired arterial baroreflex regulation of heart rate after blockade of P2-purinoceptors in the nucleus tractus solitarius. *Brain Res Bull* 47, 63-67 (1998)

37. Paton JF, De Paula PM, Spyer KM, Machado BH, Boscan P: Sensory afferent selective role of P2 receptors in the nucleus tractus solitarii for mediating the cardiac component of the peripheral chemoreceptor reflex in rats. *J Physiol* 543, 995-1005 (2002)

38. Horiuchi J, Potts PD, Tagawa T, Dampney RA: Effects of activation and blockade of P2X receptors in the ventrolateral medulla on arterial pressure and sympathetic activity. *J Auton Nerv Syst* 76, 118-126 (1999)

39. Ralevic V: P2 receptors in the central and peripheral nervous systems modulating sympathetic vasomotor tone. *J Auton Nerv Syst* 81, 205-211 (2000)

40. Spyer KM, Thomas T: Sensing arterial CO_2 levels: a role for medullary P2X receptors. *J Auton Nerv Syst* 81, 228-235 (2000)

41. Kidd EJ, Grahames CB, Simon J, Michel AD, Barnard EA, Humphrey PP: Localization of P2X purinoceptor transcripts in the rat nervous system. *Mol Pharmacol* 48, 569-573 (1995)

42. Seguela P, Haghighi A, Soghomonian JJ, Cooper E: A novel neuronal P2X ATP receptor ion channel with widespread distribution in the brain. *J Neurosci* 16, 448-455 (1996)

43. Vulchanova L, Arvidsson U, Riedl M, Wang J, Buell G, Surprenant A, North RA, Elde R: Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. *Proc. Natl. Acad. Sci. USA* 93, 8063-8067 (1996)

44. Yao ST, Barden JA, Finkelstein DI, Bennett MR, Lawrence AJ: Comparative study on the distribution patterns of $P2X_1$-$P2X_6$ receptor immunoreactivity in the brainstem of the rat and the common marmoset (*Callithrix jacchus*): association with catecholamine cell groups. *J Comp Neurol* 427, 485-507 (2000)

45. Yao ST, Barden JA, Lawrence AJ: On the immunohistochemical distribution of ionotropic P2X receptors in the nucleus tractus solitarius of the rat. *Neuroscience* 108, 673-685 (2001)

46. Boulant JA: Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clin Infect Dis* 31, Suppl 5, S157-S161 (2000)

47. Gourine AV, Rudolph K, Tesfaigzi J, Kluger MJ: Role of hypothalamic interleukin-1β in fever induced by cecal ligation and puncture in rats. *Am J Physiol Regul Integr Comp Physiol* 275, R754-R761 (1998)

48. Klir JJ, McClellan JL, Kluger MJ: Interleukin-1β causes the increase in anterior hypothalamic interleukin-6 during LPS-induced fever in rats. *Am J Physiol* 266, R1845-R1848 (1994)

49. King BF, Townsend-Nicholson A: Nucleotide and Nucleoside Receptors. In: *Tocris Reviews* 23, 1-12 (2003)

50. Latini S, Pedata F: Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem* 79, 463-484 (2001)

51. de Mendonça A, Sebastião AM, Ribeiro JA: Adenosine: does it have a neuroprotective role after all? *Brain Res Brain Res Rev* 33, 258-274 (2000)

52. Zimmermann H: Biochemistry, localization and functional roles of ecto-nucleotidases in the nervous system. *Prog Neurobiol* 49, 589-618 (1996)

53. Zimmermann H: Extracellular metabolism of ATP and other nucleotides. *Naunyn Schmiedebergs Arch Pharmacol* 362, 299-309 (2000)

54. Hasko G, Kuhel DG, Chen JF, Schwarzschild MA, Deitch EA, Mabley JG, Marton A, Szabo C: Adenosine inhibits IL-12 and TNF-α production via adenosine A_{2A} receptor-dependent and independent mechanisms. *FASEB J* 14, 2065-2074 (2000)

55. McWhinney CD, Dudley MW, Bowlin TL, Peet NP, Schook L, Bradshaw M, De M, Borcherding DR, Edwards CK 3rd: Activation of adenosine A_3 receptors on macrophages inhibits tumor necrosis factor-α. *Eur J Pharmacol* 310, 209-216 (1996)

56. Sajjadi FG, Takabayashi K, Foster AC, Domingo RC, Firestein GS: Inhibition of TNF-α expression by adenosine: role of A_3 adenosine receptors. *J Immunol* 156, 3435-3442 (1996)

57. Adanin S, Yalovetskiy IV, Nardulli BA, Sam AD 2nd, Jonjev ZS, Law WR: Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis. *Am J Physiol Regul Integr Comp Physiol* 282, R1324-R1332 (2002)

58. Hasko G, Szabo C, Nemeth ZH, Kvetan V, Pastores SM, Vizi ES: Adenosine receptor agonists differentially regulate IL-10, TNF-α, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. *J Immunol* 157, 4634-4640 (1996)

59. Hasko G, Kuhel DG, Nemeth ZH, Mabley JG, Stachlewitz RF, Virag L, Lohinai Z, Southan GJ, Salzman AL, Szabo C: Inosine inhibits inflammatory cytokine production by a posttranscriptional mechanism and protects against endotoxin-induced shock. *J Immunol* 164, 1013-1019 (2000)

60. Straub RH, Pongratz G, Gunzler C, Michna A, Baier S, Kees F, Falk W, Scholmerich J: Immunoregulation of IL-6 secretion by endogenous and exogenous adenosine and by exogenous purinergic agonists in splenic tissue slices. *J Neuroimmunol* 125, 73-81 (2002)

61. Majumdar S, Aggarwal BB: Adenosine suppresses activation of nuclear factor-kappaB selectively induced by tumor necrosis factor in different cell types. *Oncogene* 22, 1206-1218 (2003)

62. Wilson CN, Batra VK: Lipopolysaccharide binds to and activates A_1 adenosine receptors on human pulmonary artery endothelial cells. *J Endotoxin Res* 8, 263-271 (2002)

63. Reppert SM, Weaver DR, Stehle JH, Rivkees SA: Molecular cloning and characterization of a rat A_1-adenosine receptor that is widely expressed in brain and spinal cord. *Mol Endocrinol* 5, 1037-1048 (1991)

64. El Yacoubi M, Ledent C, Parmentier M, Ongini E, Costentin J, Vaugeois JM: *In vivo* labelling of the adenosine A_{2A} receptor in mouse brain using the selective antagonist [³H]SCH 58261. *Eur J Neurosci* 14, 1567-1570 (2001)

65. Strecker RE, Morairty S, Thakkar MM, Porkka-Heiskanen T, Basheer R, Dauphin LJ, Rainnie DG, Portas CM, Greene RW, McCarley RW: Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. *Behav Brain Res* 115, 183-204 (2000)

66. Thakkar MM, Winston S, McCarley RW: Orexin neurons of the hypothalamus express adenosine A_1 receptors. *Brain Res* 944: 190-194 (2002)

67. Bisserbe JC, Patel J, Marangos PJ: Autoradiographic localization of adenosine uptake sites in the brain using [³H] nitrobenzylthioinosine. *J Neurosci* 5, 544-550 (1985)

68. Lawrence AJ, Castillo-Melendez M, Mclean KJ, Jarrott B: The distribution of nitric oxide synthase-, adenosine deaminase- and neuropeptide Y-immunoreactivity through the entire rat nucleus tractus solitarius: Effect of unilateral nodose ganglionectomy. *J Chem Neuroanat* 15, 27-40 (1998)

69. St Lambert JH, Dashwood MR, Spyer KM: Role of brainstem adenosine A_1 receptors in the cardiovascular response to hypothalamic defence area stimulation in the anaesthetised rat. *Br J Pharmacol* 117, 277-282 (1996)

70. Thomas T, St Lambert JH, Dashwood MR, Spyer KM: Localization and action of adenosine A_{2a} receptors in regions of the brainstem important in cardiovascular control. *Neuroscience* 95, 513-518 (2000)

71. Thomas T, Spyer KM: A novel influence of adenosine on ongoing activity in rat rostral ventrolateral medulla. *Neuroscience* 88, 1213-1223 (1999)

72. Barraco R, Ergene E, El-Ridi M: Purinergic receptors in the nucleus tractus solitarius mediate distinct cardiorespiratory response patterns. *Drug Develop Res* 28, 309-314 (1993)

73. Eldridge FL, Millhorn DE, Kiley JP: Antagonism by theophylline of respiratory inhibition induced by adenosine. *J Appl Physiol* 59, 1428-1433 (1985)

74. Mironov SL, Langohr K, Richter DW: A_1 adenosine receptors modulate respiratory activity of the neonatal mouse via the cAMP-mediated signaling pathway. *J Neurophysiol* 81, 247-255 (1999)

75. Schmidt C, Bellingham MC, Richter DW: Adenosinergic modulation of respiratory neurones and hypoxic responses in the anaesthetized cat. *J Physiol* 483, 769-781 (1995)

76. Dawid-Milner MS, Silva-Carvalho L, Goldsmith GE, Spyer KM: A potential role of central A1 adenosine receptors in the response to hypothalamic stimulation. *J Auton Nerv Syst* 49, 15-19 (1994)

77. Spyer KM, Thomas T: A role for adenosine in modulating cardio-respiratory responses: a mini-review. *Brain Res Bull* 53, 121-124 (2000)

78. St Lambert JH, Thomas T, Burnstock G, Spyer KM: A source of adenosine involved in cardiovascular responses to defence area stimulation. *Am J Physiol* 272, R195-R200 (1997)

79. Dale N, Gourine AV, Llaudet E, Bulmer D, Thomas T, Spyer KM: Rapid adenosine release in the nucleus tractus solitarii during defence response in rats: real-time measurement *in vivo*. *J Physiol* 544, 149-160 (2002)

80. Romanovsky AA, Simons CT, Székely M, Kulchitsky VA: The vagus nerve in the thermoregulatory response to systemic inflammation. *Am J Physiol* 273, R407-R413 (1997)

81. Sehic E, Blatteis CM: Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea pigs. *Brain Res* 726, 160-166 (1996)

82. Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, Maier SF: Blockade of interleukin-1-induced hyperthermia by subdiaphragmatic vagotomy: Evidence for vagal mediation of immune-to-brain communication. *Neurosci Lett* 183, 27-31 (1995)

83. Ticho SR, Radulovacki M: Role of adenosine in sleep and temperature regulation in the preoptic area of rats. *Pharmacol Biochem Behav* 40, 33-40 (1991)

84. Mehta AK, Kulkarni SK: Effect of purinergic substances on rectal temperature in mice: involvement of P1-purinoceptors. *Arch Int Pharmacodyn Ther* 264, 180-186 (1983)

85. Anderson R, Sheehan MJ, Strong P: Characterization of the adenosine receptors mediating hypothermia in the conscious mouse. *Br J Pharmacol* 113, 1386-1390 (1994)

86. Matuszek MT, Gagalo IT: The antipyretic activity of adenosine agonists. *Gen Pharmacol* 29, 371-374 (1997)

87. Branco LG, Steiner AA, Tattersall GJ, Wood SC: Role of adenosine in the hypoxia-induced hypothermia of toads. *Am J Physiol Regul Integr Comp Physiol* 279, R196-201 (2000)

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