British Journal of Pharmacology (2005) 146, 139–145
www.nature.com/bjp

P2 receptor blockade attenuates fever and cytokine responses induced by lipopolysaccharide in rats

*,[1]Alexander V. Gourine, [2]Dmitry M. Poputnikov, [2]Nikolai Zhernosek, [2]Ekaterina V. Melenchuk, [3]Rüdiger Gerstberger, [1]K. Michael Spyer & [2]Valery N. Gourine

[1]Department of Physiology, Royal Free and University College London Medical School, University College London, Hampstead Campus, Rowland Hill Street, London NW3 2PF; [2]Institute of Physiology, National Academy of Sciences of Belarus, Minsk 220725, Belarus and [3]Institut für Veterinär-Physiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

1 Adenosine 5′-triphosphate (ATP) has been shown to induce release of cytokines implicated in fever, including interleukin(IL)-1β, IL-6, and tumour necrosis factor-α (TNF-α). The role of ATP-mediated purinergic signalling in fever and cytokine release during systemic inflammation was investigated by studying the effects of P2 receptor antagonists suramin, pyridoxal-5′-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), and Brilliant Blue G (BBG) on changes in body temperature and the increases in plasma levels of IL-1β, IL-6, and TNFα induced by bacterial lipopolysaccharide (LPS) in rats.

2 LPS (*Escherichia coli*; 50 μg kg^{-1})-induced febrile response was attenuated by suramin (25 mg kg^{-1} and 100 mg kg^{-1}), PPADS (25 mg kg^{-1}), and a more selective $P2X_7$ receptor antagonist BBG (100 mg kg^{-1}) injected intraperitoneally before the induction of fever.

3 The increase in plasma concentrations of IL-1β and IL-6, measured 1 h after LPS treatment, was reduced by PPADS (25 mg kg^{-1}) and BBG (100 mg kg^{-1}). LPS-induced increase in plasma TNF-α concentration was also markedly attenuated by BBG (100 mg kg^{-1}), but not by PPADS (25 mg kg^{-1}).

4 These data indicate that purinergic signalling plays an important role in the mechanisms responsible for the LPS-induced febrile response and increases in the levels of circulating cytokines. We suggest that ATP acting *via* $P2X_7$ receptors induces release of pyrogenic cytokines to mediate fever during systemic inflammation.
British Journal of Pharmacology (2005) **146,** 139–145. doi:10.1038/sj.bjp.0706287;
published online 20 June 2005

Keywords: ATP; body temperature; Brilliant Blue G; fever; interleukin-1β; interleukin-6; $P2X_7$ receptors; pyridoxal-5′-phosphate-6-azophenyl-2′,4′-disulphonic acid; suramin; tumour necrosis factor-α

Abbreviations: ANOVA, analysis of variance; ATP, adenosine 5′-triphosphate; BBG, Brilliant Blue G; i.p., intraperitoneal; IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; PPADS, pyridoxal-5′-phosphate-6-azophenyl-2′,4′-disulphonic acid; s.e., standard error; T_b, body temperature; TNF-α, tumour necrosis factor-α

Introduction

Systemic inflammation during infection is accompanied by a number of specific behavioural and autonomic adaptive responses aimed at facilitating host resistance and slowing the growth of the pathogen. Fever – a regulated rise in body temperature (T_b) – is one of these adaptive responses.

There is ample of evidence indicating that circulating cytokines, such as interleukin (IL)-1β, IL-6, tumour necrosis factor-α (TNF-α), and others, act as endogenous pyrogens and play an important role in the mechanisms responsible for the development of the febrile response during infection and inflammation (Kluger, 1991; Kluger *et al*., 1995; Conti *et al*., 2004). Several potential mechanisms of cytokine involvement in fever have been proposed including their peripheral (e.g. activation of the subdiaphragmatic afferent fibres of the vagus nerve, which is believed to be responsible for the induction of the febrile response) and central (e.g. penetration into the brain or production within the brain and modification of the activity of temperature-sensitive neurones in the preoptic hypothalamic region involved in thermoregulation) action (for a recent review, see Conti *et al*., 2004). At present, there is a general consensus that circulating cytokines are essential for the development of the febrile response in inflammatory conditions. Taking into the account the central role of cytokines in fever, we proposed earlier (Gourine *et al*., 2002a) that any endogenous factor involved in the mechanisms of cytokine production and/or clearance may also play an important role in fever.

The role of adenosine 5′-triphosphate (ATP)-mediated purinergic signalling in the immunological and inflammatory responses have been under intense scrutiny in recent years (Dubyak, 2000; la Sala *et al*., 2003). Particular attention has been paid to the close interaction between extracellular ATP and cytokines. In different *in vitro* models ATP has been shown to induce release of IL-1β (Perregaux & Gabel, 1998, Perregaux *et al*., 2000; Mehta *et al*., 2001; Chakfe *et al*., 2002), IL-6 (Inoue, 2002), IL-8 (Idzko *et al*., 2003), IL-18 (Mehta *et al*., 2001), and TNF-α (Hide *et al*., 2000; Suzuki *et al*., 2004; Kucher & Neary, 2005) by various cell types, including monocytes/macrophages, microglial cells, and others. There

*Author for correspondence; E-mail: a.gourine@rfc.ucl.ac.uk

is evidence that ATP induces cytokine release by these cells through action at ionotropic $P2X_7$ receptors (Hide *et al*., 2000; Mehta *et al*., 2001; Solle *et al*., 2001; Chakfe *et al*., 2002; North, 2002, Colomar *et al*., 2003; Idzko *et al*., 2003; Suzuki *et al*., 2004). In mice lacking $P2X_7$ receptors, peritoneal macrophages are unable to release IL-1β in response to ATP (Solle *et al*., 2001) while collagen-induced arthritis results in a significantly attenuated systemic inflammatory response (Labasi *et al*., 2002).

The evidence that ATP, acting *via* $P2X_7$ receptors, induces production of cytokines suggested that purinergic signalling may play an important role in cytokine release and the development of the febrile response during infection and inflammation. However, most of the studies mentioned above only described the effect of ATP on cytokine production by different immune cells *in vitro*. The functional role of ATP in '*in vivo*' cytokine release remains largely unknown. In this study, experiments have been designed to investigate the putative role of ATP-mediated purinergic signalling in febrile and cytokine responses during systemic inflammation. This role was investigated by determining the effects of P2 receptor antagonists suramin, pyridoxal-5′-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), and Brilliant Blue G (BBG, selective $P2X_7$ receptor blocker) on changes in T_b and increases in plasma levels of IL-1β, IL-6, and TNFα induced by bacterial endotoxin lipopolysaccharide (LPS) in rats. Some of the results obtained have been reported previously in a preliminary abstract (Gourine *et al*., 2004).

Methods

Animals

Adult male Wistar rats weighing 280–320 g were used in this study. They were housed in a room maintained at a constant temperature of $25 \pm 1^{\circ}C$ and in a 12 : 12 h light–dark cycle with light onset at 0600 hours. Drinking water and Laboratory Rodent Chow were provided *ad libitum*. All studies on conscious rats were conducted in facilities of the Institute of Physiology, National Academy of Sciences of Belarus and were approved by the Institutional Animal Care and Use Committee.

Surgery

Rats were anaesthetised with a mixture of ketamine hydrochloride ($87.0\,mg\,kg^{-1}$) and xylazine hydrochloride ($13.0\,mg\,kg^{-1}$) injected intramuscularly. A miniature temperature-sensitive telemetry transmitter (model E-mitter, Minimitter, Sunriver, OR, U.S.A.) was implanted into the abdominal cavity of each rat for continuous monitoring of T_b. After the surgery, animals were housed one per cage and were allowed to recover for at least 7 days before any experiment. At the end of the experiment, the animals were killed humanely by an overdose of pentobarbitone sodium ($200\,mg\,kg^{-1}$) injected intraperitoneally (i.p.).

T_b *measurements*

Deep T_b ($\pm 0.1^{\circ}C$) was monitored with implanted telemetry units (Minimitter). Recordings were made at 1-min intervals by the use of a peripheral processor (VitalView System, Minimitter) connected to an IBM PC.

Induction of fever

Purified LPS (*Escherichia coli* endotoxin 0111 : B4, Sigma Chemical, St Louis, MO, U.S.A.) was dissolved in pyrogen-free saline and injected i.p. at a dose of $50\,\mu g\,kg^{-1}$ and in a volume of ~ 0.3 ml. Control rats received an equivalent volume of sterile pyrogen-free saline.

Drugs

As selective rat $P2X_7$ receptor antagonists are not readily available (Baraldi *et al*., 2004), we used the generic P2 blockers suramin and PPADS as well as the compound widely used to antagonize $P2X_7$ receptors – BBG. Currents evoked at rat $P2X_7$ receptors are to some extent sensitive to block by PPADS ($IC_{50} \sim 50\,\mu M$) and suramin ($IC_{50} > 300\,\mu M$) (Surprenant *et al*., 1996). BBG is significantly more potent and produces a noncompetitive inhibition of the rat $P2X_7$ receptors with $IC_{50} \sim 10$ nM (Jiang *et al*., 2000). The other subtypes of the rat P2X receptors are blocked in the micromolar range or unaffected by BBG (Jiang *et al*., 2000). PPADS, suramin, and BBG were obtained from Sigma Chemical and were dissolved in saline for injections.

ELISA

Blood for cytokine analysis was collected rapidly from terminally anaesthetised (pentobarbitone sodium, $100\,mg\,kg^{-1}$, i.p.) rats by cardiac puncture. Blood was drawn into heparinised syringes and plasma was separated by centrifugation (12,000 r.p.m., 5 min, 20°C) of the freshly drawn blood and stored at −20°C until assayed. Cytokines were not separated from the plasma carrier proteins before assays. IL-1β, IL-6, and TNFα concentrations in plasma were measured using rat-IL-1β, rat-IL-6, and rat-TNFα immunoassay kits (R&D Systems, Minneapolis, U.S.A.) according to the manufacturer's instructions. These assays detect IL-1β, IL-6, and TNFα at concentrations as low as $5\,pg\,ml^{-1}$, $14\,pg\,ml^{-1}$, and $5\,pg\,ml^{-1}$, respectively.

Experimental design

Experiment 1. Effect of the generic P2 receptor antagonist suramin on T_b *during LPS-induced fever in rats* Suramin ($5\,mg\,kg^{-1}$ ($n=7$), $25\,mg\,kg^{-1}$ ($n=9$), or $100\,mg\,kg^{-1}$ ($n=11$)) was injected i.p. 5 min prior to LPS administration and the effect of this treatment on the development of fever in conscious freely moving rats was determined. Control animals ($n=10$) received an equivalent volume of sterile pyrogen-free saline 5 min before LPS injection. There were two additional control groups of animals: six rats injected i.p. with suramin ($100\,mg\,kg^{-1}$) and saline, and nine rats that received two successive i.p. injections of saline. T_b was monitored for 2 h before and 9 h after the injections, which were performed between 0900 and 1000 hours.

Experiment 2. Effect of the generic P2 receptor antagonist PPADS on T_b *during LPS-induced fever in rats* The design of this experiment was similar to the *Experiment 1*. In conscious freely moving rats, PPADS ($5\,mg\,kg^{-1}$ ($n=6$), or $25\,mg\,kg^{-1}$ ($n=12$)) or sterile pyrogen-

free saline ($n=12$) were given i.p. 5 min prior to LPS treatment. Nine rats were injected i.p. with PPADS (25 mg kg^{-1}) and saline, and 10 rats received two successive i.p. injections of saline.

Experiment 3. Effect of P2X$_7$ receptor blocker BBG on T$_b$ during LPS-induced fever in rats The design of this experiment was similar to the *Experiments 1 and 2*. The effect of BBG (40 mg kg^{-1} ($n=6$), or 100 mg kg^{-1} ($n=6$)) on LPS-induced fever in conscious freely moving rats was determined.

Experiment 4. Effect of the generic P2 receptor antagonist PPADS and P2X$_7$ receptor blocker BBG on LPS-induced increases in plasma levels of IL-1β, IL-6, and TNF-α in rats From two generic P2 receptor antagonists used in this study, PPADS was chosen for this experiment because it is a more selective antagonist of P2 receptors than suramin. In addition, PPADS is significantly more potent than suramin in terms of blockade of P2X$_7$ receptors (Surprenant *et al*., 1996; North, 2002). The effect of PPADS on LPS-induced increases in plasma levels of IL-1β, IL-6, and TNF-α was compared to that of BBG – the most potent rat P2X$_7$ receptor antagonist that is readily available (Jiang *et al*., 2000). Plasma concentrations of IL-1β, IL-6, and TNF-α were measured at 1 h after injection of LPS because at this time point high plasma levels of all these cytokines are observed, while TNFα level is at or near its peak (Waage, 1987; Chensue *et al*., 1991).

In a separate experiment conscious rats were injected i.p. with PPADS (25 mg kg^{-1}) or BBG (100 mg kg^{-1}) 5 min prior to LPS administration. Control animals received an equivalent volume of pyrogen-free saline 5 min before LPS injection. There were two additional control groups of animals in each experiment: rats injected i.p. with PPADS or BBG followed by injection of saline, and rats that received two successive i.p. injections of saline. Blood was collected from terminally anaesthetised animals by cardiac puncture 1 h after LPS administration.

Statistical analysis

Data are reported as mean ± standard error (s.e.). The T_b data were analysed using two-way analysis of variance (ANOVA) with Tukey's *post hoc* test to determine main group and time effects. IL-1β, IL-6, and TNF-α levels in plasma were compared by one-way ANOVA followed by the *post hoc* Fisher's test. Values of $P<0.05$ were considered to be significant.

Results

Experiment 1. Effect of the generic P2 receptor antagonist suramin on T$_b$ during LPS-induced fever in rats Following i.p. injection of LPS, fever reached a maximal T_b (around 39°C) ~2.5 h after the injection ($P<0.01$, compared to saline-treated controls) (Figures 1–3). Suramin (5 mg kg^{-1}) injected i.p. 5 min prior to LPS administration had no significant effect on the febrile response (Figure 1a). However, LPS-induced fever was significantly attenuated when suramin was given at a dose of 25 mg kg^{-1} ($P<0.05$, compared to the saline/LPS group; Figure 1b). LPS failed to induce fever in rats pretreated i.p. with suramin at a dose of 100 mg kg^{-1} ($P<0.01$, compared to the saline/LPS group; Figure 1c). No significant changes in T_b were evoked by suramin in afebrile animals. The T_b of rats injected with suramin (100 mg kg^{-1}) and saline was similar to that of rats injected with saline and saline (Figure 1c).

Experiment 2. Effect of the generic P2 receptor antagonist PPADS on T$_b$ during LPS-induced fever in rats I.p. injection of PPADS at a dose of 5 mg kg^{-1} had no significant effect on LPS-induced fever (Figure 2a). However, PPADS administered at a dose of 25 mg kg^{-1} attenuated significantly the febrile response evoked by LPS ($P<0.05$, compared to the saline/LPS group; Figure 2b). No significant changes in T_b were induced by PPADS in afebrile animals. The

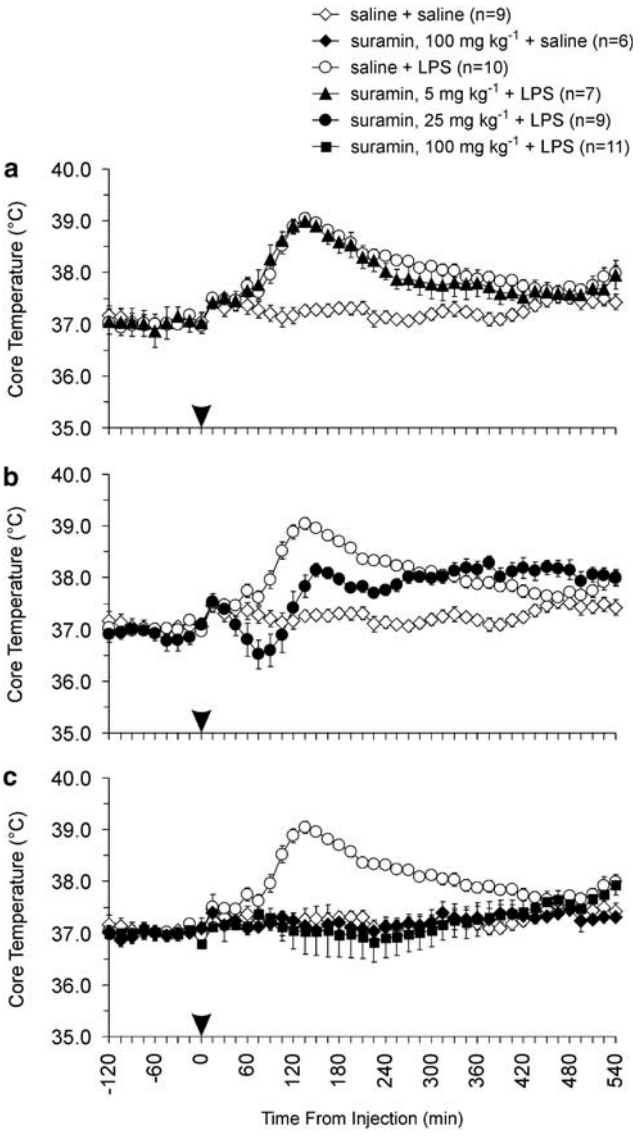

Figure 1 Effect of the P2 receptor antagonist suramin on T_b during fever induced by *E. coli* lipopolysaccharide (LPS, 50 μg kg^{-1}) in rats. Suramin (5 mg kg^{-1} (a), 25 mg kg^{-1} (b), or 100 mg kg^{-1} (c)) was injected i.p. 5 min prior to LPS administration. Data are presented as means ± s.e. Numbers in parentheses indicate sample sizes. Arrowhead indicates time of injections.

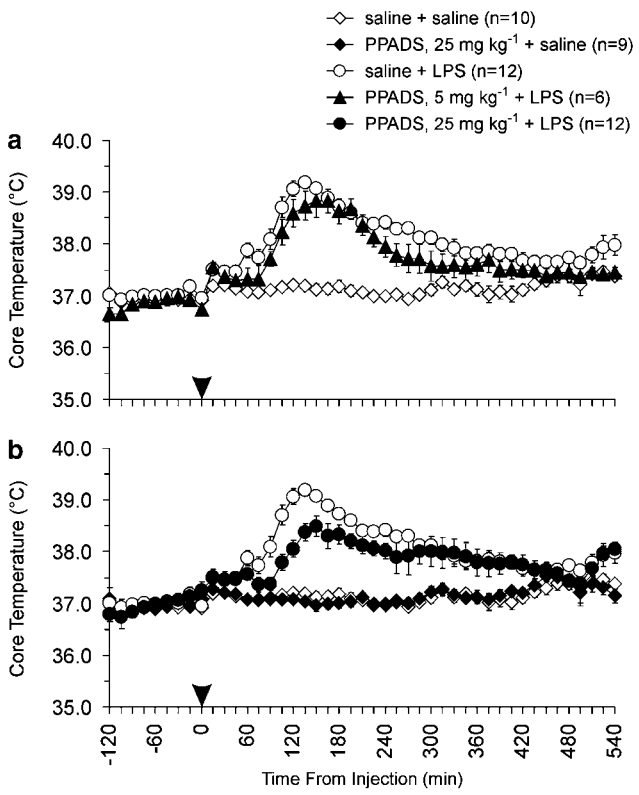

Figure 2 Effect of the P2 receptor antagonist pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) on T_b during fever induced by *E. coli* lipopolysaccharide (LPS, 50 μg kg^{-1}) in rats. PPADS (5 mg kg^{-1} (a), or 25 mg kg^{-1} (b) was injected i.p. 5 min prior to LPS administration. Data are presented as means ± s.e. Numbers in parentheses indicate sample sizes. Arrowhead indicates time of injections.

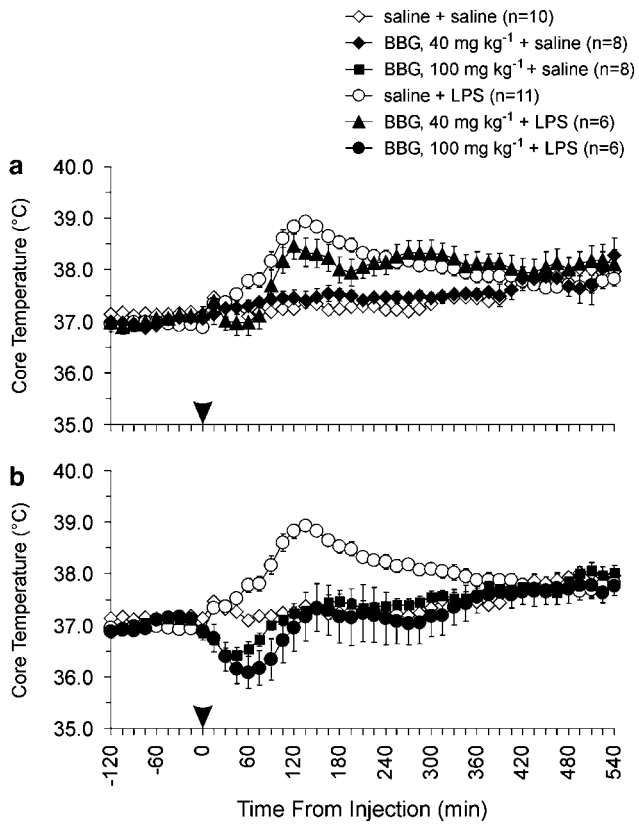

Figure 3 Effect of the P2X$_7$ receptor blocker Brilliant Blue G (BBG) on T_b during fever induced by *E. coli* lipopolysaccharide (LPS, 50 μg kg^{-1}) in rats. BBG (40 mg kg^{-1} (a), or 100 mg kg^{-1} (b) was injected i.p. 5 min prior to LPS administration. Data are presented as means ± s.e. Numbers in parentheses indicate sample sizes. Arrowhead indicates time of injections.

T_b of rats treated with PPADS (25 mg kg^{-1}) and saline was similar to that of rats which received two successive injections of saline (Figure 2b).

Experiment 3. Effect of P2X$_7$ receptor blocker BBG on T_b *during LPS-induced fever in rats* BBG injected at a dose of 40 mg kg^{-1} appeared to slightly attenuate the early LPS-induced increases in T_b (Figure 3a); however, the differences in the overall febrile response between BBG (40 mg kg^{-1})/LPS and saline/LPS groups failed to reach statistical significance ($P>0.05$). However, LPS-induced fever was blocked completely when BBG was given at a dose of 100 mg kg^{-1} ($P<0.01$, compared to the saline/LPS group; Figure 2b). Note that BBG at this dose (100 mg kg^{-1}) evoked a marked ($\sim$1°C) decrease in T_b shortly after injection (Figure 3b). A similar decrease in the resting T_b was evoked by BBG (100 mg kg^{-1}) in afebrile rats ($P<0.05$, compared to the saline/saline group; Figure 3b). Note that the T_b of rats injected with BBG (100 mg kg^{-1}) and LPS was similar to that of rats injected with BBG and saline (Figure 3b) – indicating complete blockade of the febrile response by the action of BBG.

*Experiment 4. Effect of the generic P2 receptor antagonist PPADS and P2X$_7$ receptor blocker BBG on LPS-induced increases in plasma levels of IL-1*β*, IL-6, and TNF-*α *in rats* Plasma IL-1β, IL-6, and TNF-α levels were measured in rats treated with either PPADS or BBG at 1 h following injection of LPS or saline (Figures 4 and 5). LPS injection resulted in a profound and rapid elevation in plasma levels of all cytokines. BBG (100 mg kg^{-1}) significantly attenuated while PPADS (25 mg kg^{-1}) completely blocked LPS-induced increase in plasma level of IL-1β (Figures 4 and 5). Similarly, concentration of IL-6 in plasma of rats injected with PPADS or BBG followed by LPS treatment was significantly ($P<0.05$) lower compared to that of rats injected with saline and LPS (Figures 4 and 5). LPS-induced increase in plasma TNF-α concentration was also dramatically attenuated by BBG ($P<0.05$; Figure 5), but not by PPADS (Figure 4).

Discussion

The data obtained in the present study indicate that ATP-mediated purinergic signalling plays an important role in the mechanisms responsible for the febrile response and increases in the levels of circulating cytokines during systemic inflammation. Considering the central role of IL-1β, IL-6, and TNF-α in fever, we suggest that ATP induces release of pyrogenic cytokines by the cells of the immune system to mediate the febrile response during systemic inflammation.

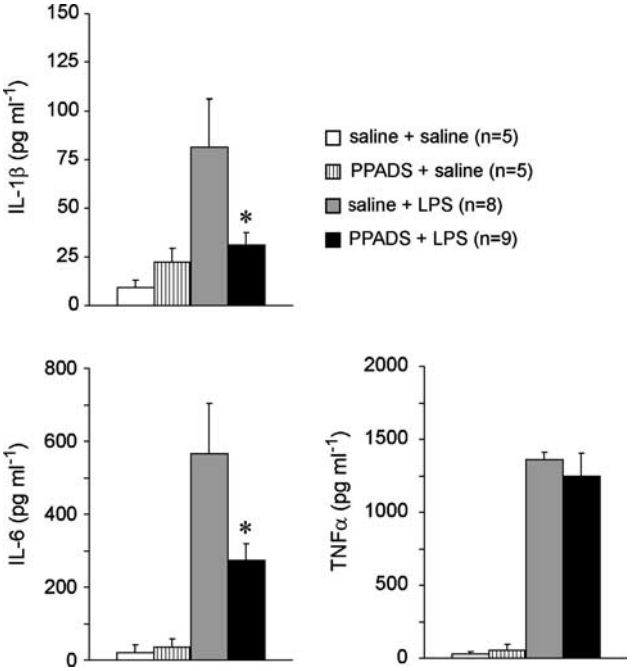

Figure 4 Effect of the P2 receptor antagonist pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) on interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-α (TNFα) concentrations in blood plasma during the development of fever induced by *E. coli* lipopolysaccharide (LPS, 50 μg kg^{-1}) in rats. PPADS (25 mg kg^{-1}) was injected i.p. 5 min prior to LPS administration. Plasma IL-1β, IL-6, and TNFα levels were measured at 60 min following injection of LPS or saline. Data are presented as means $\pm$ s.e. Numbers in parentheses indicate sample sizes. *Significant difference between LPS-treated groups (saline + LPS vs PPADS + LPS), $P<0.05$.

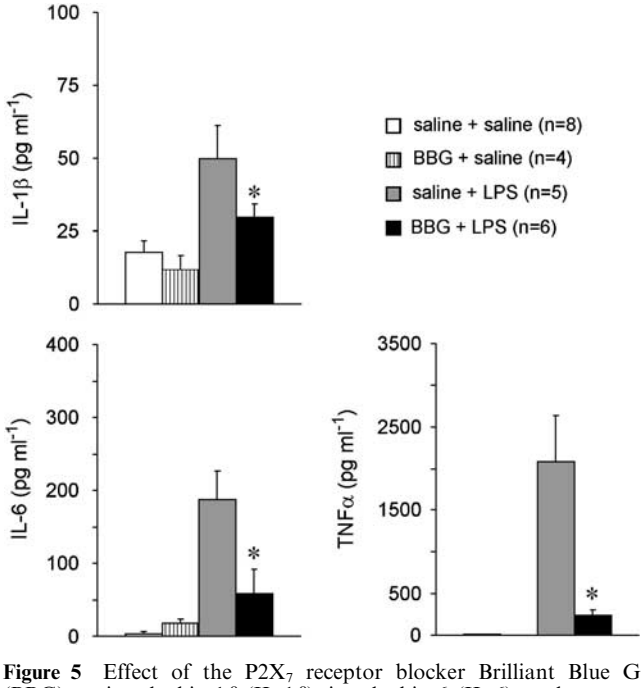

Figure 5 Effect of the P2X$_7$ receptor blocker Brilliant Blue G (BBG) on interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-α (TNFα) concentrations in blood plasma during the development of fever induced by *E. coli* lipopolysaccharide (LPS, 50 μg kg^{-1}) in rats. BBG (100 mg kg^{-1}) was injected i.p. 5 min prior to LPS administration. Plasma IL-1β, IL-6, and TNFα levels were measured at 60 min following injection of LPS or saline. Data are presented as means $\pm$ s.e. Numbers in parentheses indicate sample sizes. *Significant difference between LPS-treated groups (saline + LPS vs BBG + LPS), $P<0.05$.

Taking into the account the evidence that ATP induces cytokine release by acting at ionotropic P2X$_7$ receptors, we suggested initially that this mechanism may have an important role in the development of cytokine and febrile responses during inflammatory process. To test this hypothesis, P2X$_7$ receptors had to be blocked and the effect of this blockade on changes in T_b and plasma cytokine levels during the immune challenge had to be determined. However, the lack of selective P2X$_7$ receptor antagonists is the major caveat in the studies of the functional role of ATP-mediated purinergic signalling at 'immunological synapses' (Baraldi *et al.*, 2004).

Here, we used two generic P2 receptor blockers suramin and PPADS as well as the compound widely used to antagonize rat P2X$_7$ receptors – BBG. As mentioned above, rat P2X$_7$ receptors in the expression systems are to some extent sensitive to blockade by both PPADS ($IC_{50} \sim 50\,\mu$M) and suramin ($IC_{50} > 300\,\mu$M) (Surprenant *et al.*, 1996). Thus, PPADS appears to be significantly more potent than suramin in terms of P2X$_7$ receptor blockade. There is, however, evidence showing that suramin blocks effectively native P2X$_7$ receptors in human lymphocytes (Wiley *et al.*, 1993). The third compound used in this study – BBG – produces a non-competitive inhibition of the rat P2X$_7$ receptors with $IC_{50} \sim 10$ nM (Jiang *et al.*, 2000). The other subtypes of the rat P2X receptors are blocked in the micromolar range or unaffected by BBG (Jiang *et al.*, 2000).

All three antagonists (suramin, PPADS, and BBG) attenuated (although to a different extent) the febrile response evoked by systemic treatment with LPS. The exact duration of the P2 receptor blockade and the actual concentration of the drugs at their target sites (e.g. liver – one of the major sources of circulating cytokines) following i.p. injections of suramin, PPADS, or BBG are difficult to estimate. Note that suramin and BBG in a high dose completely blocked any LPS-induced increases in T_b. Obviously, when administered in a high dose, the duration of action of these antagonists and their concentrations at target sites are sufficient to ablate the whole febrile response. However, both compounds have other actions in addition to their ability to block P2 receptors (e.g. BBG is known for its ability to bind various proteins). Note a decrease in resting T_b in afebrile rats following administration of BBG. PPADS is more selective, but it produced only partial inhibition of the febrile response. Considering together the data from the use of suramin, PPADS, and BBG the hypothesis that attenuation of fever following administration of P2 receptor antagonists could be due to the inhibition of P2X$_7$ receptors is sustained.

The mechanisms responsible for a decrease in resting T_b in afebrile rats in response to BBG are unknown. We also observed a slight decrease in T_b below the baseline in the experimental group treated with suramin (25 mg kg^{-1}) and LPS (although, suramin in a significantly higher dose of 100 mg kg^{-1} did not affect resting T_b in afebrile rats). These decreases in T_b are unlikely to be mediated by the action of these compounds within the central nervous system. We have shown previously that P2 receptor antagonists injected into the

brain induce increase in T_b in afebrile rats and facilitate fever in rats treated with LPS (Gourine *et al*., 2002b). Thus, it is most likely that the action of P2 receptor antagonists on as yet unidentified peripheral targets may trigger a decrease in normal T_b.

Both PPADS and BBG attenuated significantly increases in plasma levels of IL-1β and IL-6 during systemic inflammation. Interestingly, the increase in plasma level of TNF-α was blocked only by BBG, while PPADS was without any effect. This was rather unexpected, because PPADS was found to effectively block any LPS-induced increase in plasma level of the other key proinflammatory cytokine – IL-1β. Presumably, the highest dose of PPADS used ($25\,mg\,kg^{-1}$) was still insufficient to influence the release of TNF-α induced by LPS. Note that treatment with BBG resulted in a complete blockade of the LPS-induced fever, while administration of PPADS ($25\,mg\,kg^{-1}$) just attenuated the febrile response. It is possible, therefore, that TNF-α may be responsible for this residual rise in T_b at the time when LPS-induced production and (or) release of pyrogenic IL-1β and IL-6 was diminished by PPADS.

In an earlier study from our laboratories, we demonstrated that extracellular ATP, acting through as yet unidentified P2 receptors, is involved in the brain mechanisms of T_b regulation (Gourine *et al*., 2002b). We suggested that ATP modulates transmission in the hypothalamic neural pathway from peripheral warm-receptors to the neurones responsible for the stimulation of heat loss and proposed that hypothalamic warm-sensitive neurones are the likely site of ATP action in relation to regulation of T_b (Gourine *et al*., 2002b; 2004).

From the results presented here it appears that ATP-mediated purinergic signalling in the periphery plays an important role in the mechanisms of the febrile response during systemic inflammation. This action of ATP is likely to be achieved *via* $P2X_7$ receptor-mediated production and (or) release of pyrogenic cytokines. It is well known that activated immune cells such as lymphocytes, macrophages, and others release large amounts of ATP into the extracellular space (Filippini *et al*., 1990; Dubyak & el-Moatassim, 1993; Beigi *et al*., 1999; Sikora *et al*., 1999). Marked increase in extracellular ATP concentrations under inflammatory conditions has been shown *in vivo* (Lazarowski *et al*., 2000). Considering the crucial role of IL-1β, IL-6, and TNF-α in fever, we suggest that during systemic inflammation ATP released by the activated immune cells induces release of pyrogenic cytokines to mediate the febrile response. The data obtained in the *in vitro* models demonstrating that ATP is a potent inducer of all cytokines implicated in fever (see Introduction), as well as the results from the current study strongly support this hypothesis.

There is, however, evidence that blockade of cytokine (IL-1β and TNF-α) release or action often results in attenuation of the late phase of the febrile response, while the earlier LPS-induced increases in T_b are not affected (for a review, see Roth & de Souza, 2001). These data suggest that circulating cytokines are more important for maintenance rather then for induction of fever. In the present study, the whole LPS-induced febrile response was completely blocked by suramin or BBG injected in the dose of $100\,mg\,kg^{-1}$. However, P2 receptor antagonists administered in lower doses tended to attenuate the earlier phase of the LPS-induced febrile response. Thus, if circulating cytokines are indeed predominantly responsible for maintenance rather then early induction of the febrile response, then in addition to the blockade of cytokine production and (or) release, P2 receptor antagonists also block the mechanisms, responsible for early initiation of fever. It is believed that rapid activation of the subdiaphragmatic afferent fibres of the vagus nerve may be responsible for the induction of the febrile response (for a review, see Romanovsky, 2004). If so, it would be of a great interest to determine in a separate study whether release of ATP contributes to the LPS-induced changes in the activity of the abdominal vagal afferents.

Nevertheless, from the data obtained in the present study, we suggest that compounds that selectively block $P2X_7$ receptors could potentially be used in the future as antipyretic and antiinflammatory drugs in cases when high fever and (or) excessive production of proinflammatory cytokines is harmful for the host.

This study was supported by The Wellcome Trust and International Association for the promotion of cooperation with scientists from the New Independent States of the former Soviet Union (INTAS).

References

BARALDI, P.G., DI VIRGILIO, F. & ROMAGNOLI, R. (2004). Agonists and antagonists acting at $P2X_7$ receptor. *Curr. Top. Med. Chem.,* **4,** 1707–1717.

BEIGI, R., KOBATAKE, E., AIZAWA, M. & DUBYAK, G.R. (1999). Detection of local ATP release from activated platelets using cell surface-attached firefly luciferase. *Am. J. Physiol.,* **276,** C267–C278.

CHAKFE, Y., SEGUIN, R., ANTEL, J.P., MORISSETTE, C., MALO, D., HENDERSON, D. & SEGUELA, P. (2002). ADP and AMP induce interleukin-1β release from microglial cells through activation of ATP-primed $P2X_7$ receptor channels. *J. Neurosci.,* **22,** 3061–3069.

CHENSUE, S.W., TEREBUH, P.D., REMICK, D.G., SCALES, W.E. & KUNKEL, S.L. (1991). *In vivo* biologic and immunohistochemical analysis of interleukin-1α, -β and tumor necrosis factor during experimental endotoxemia. Kinetics, Kupffer cell expression, and glucocorticoid effects. *Am. J. Pathol.,* **138,** 395–402.

COLOMAR, A., MARTY, V., MEDINA, C., COMBE, C., PARNET, P. & AMEDEE, T. (2003). Maturation and release of interleukin-1β by lipopolysaccharide-primed mouse Schwann cells require the stimulation of $P2X_7$ receptors. *J. Biol. Chem.,* **278,** 30732–30740.

CONTI, B., TABAREAN, I., ANDREI, C. & BARTFAI, T. (2004). Cytokines and fever. *Front. Biosci.,* **9,** 1433–1449.

DUBYAK, G.R. (2000). Purinergic signaling at immunological synapses. *J. Auton. Nerv. Syst.,* **81,** 64–68.

DUBYAK, G.R. & EL-MOATASSIM, C. (1993). Signal transduction *via* P2-purinergic receptors for extracellular ATP and other nucleotides. *Am. J. Physiol.,* **265,** C577–C606.

FILIPPINI, A., TAFFS, R.E., AGUI, T. & SITKOVSKY, M.V. (1990). Ecto-ATPase activity in cytolytic T-lymphocytes. Protection from the cytolytic effects of extracellular ATP. *J. Biol. Chem.,* **265,** 334–340.

GOURINE, A.V., DALE, N., GOURINE, V.N. & SPYER, K.M. (2004). Fever in systemic inflammation: roles of purines. *Front. Biosci.,* **9,** 1011–1022.

GOURINE, A.V., GOURINE, V.N., TESFAIGZI, Y., CALUWAERTS, N., VAN LEUVEN, F. & KLUGER, M.J. (2002a). Role of α_2-macroglobulin in fever and cytokine responses induced by lipopolysaccharide in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **283,** R218–R226.

GOURINE, A.V., MELENCHUK, E.V., POPUTNIKOV, D.M., GOURINE, V.N. & SPYER, K.M. (2002b). Involvement of purinergic signalling in central mechanisms of body temperature regulation in rats. *Br. J. Pharmacol.,* **135,** 2047–2055.

GOURINE, V.N., POPUTNIKOV, D.M., MELENCHUK, E.V., GOURINE, A.V. & SPYER, K.M. (2004). Systemic P2 receptor blockade attenuates increase in body temperature evoked by lipopolisaccharide in conscious rats. *J. Physiol.,* **555P,** PC9.

HIDE, I., TANAKA, M., INOUE, A., NAKAJIMA, K., KOHSAKA, S., INOUE, K. & NAKATA, Y. (2000). Extracellular ATP triggers tumor necrosis factor-α release from rat microglia. *J. Neurochem.,* **75,** 965–972.

IDZKO, M., PANTHER, E., BREMER, H.C., SORICHTER, S., LUTTMANN, W., VIRCHOW, C.J., DI VIRGILIO, F., HEROUY, Y., NORGAUER, J. & FERRARI, D. (2003). Stimulation of P2 purinergic receptors induces the release of eosinophil cationic protein and interleukin-8 from human eosinophils. *Br. J. Pharmacol.,* **138,** 1244–1250.

INOUE, K. (2002). Microglial activation by purines and pyrimidines. *Glia,* **40,** 156–163.

JIANG, L.H., MACKENZIE, A.B., NORTH, R.A. & SURPRENANT, A. (2000). Brilliant Blue G selectively blocks ATP-gated rat $P2X_7$ receptors. *Mol. Pharmacol.,* **58,** 82–88.

KLUGER, M.J. (1991). Fever: role of pyrogens and cryogens. *Physiol. Rev.,* **71,** 93–127.

KLUGER, M.J., KOZAK, W., LEON, L.R., SOSZYNSKI, D. & CONN, C.A. (1995). Cytokines and fever. *Neuroimmunomodulation,* **2,** 216–223.

KUCHER, B.M. & NEARY, J.T. (2005). Bi-functional effects of ATP/P2 receptor activation on tumor necrosis factor-α release in lipopolysaccharide-stimulated astrocytes. *J. Neurochem.,* **92,** 525–535.

LABASI, J.M., PETRUSHOVA, N., DONOVAN, C., MCCURDY, S., LIRA, P., PAYETTE, M.M., BRISSETTE, W., WICKS, J.R., AUDOLY, L. & GABEL, C.A. (2002). Absence of the $P2X_7$ receptor alters leukocyte function and attenuates an inflammatory response. *J. Immunol.,* **168,** 6436–6445.

LA SALA, A., FERRARI, D., DI VIRGILIO, F., IDZKO, M., NORGAUER, J. & GIROLOMONI, G. (2003). Alerting and tuning the immune response by extracellular nucleotides. *J. Leukoc. Biol.,* **73,** 339–343.

LAZAROWSKI, E.R., BOUCHER, R.C. & HARDEN, T.K. (2000). Constitutive release of ATP and evidence for major contribution of ecto-nucleotide pyrophosphatase and nucleoside diphosphokinase to extracellular nucleotide concentrations. *J. Biol. Chem.,* **275,** 31061–31068.

MEHTA, V.B., HART, J. & WEWERS, M.D. (2001). 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.

NORTH, R.A. (2002). Molecular physiology of P2X receptors. *Physiol. Rev.,* **82,** 1013–1067.

PERREGAUX, D.G. & GABEL, C.A. (1998). 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.

PERREGAUX, D.G., McNIFF, P., LALIBERTE, R., CONKLYN, M. & GABEL, C.A. (2000). ATP acts as an agonist to promote stimulus-induced secretion of IL-1β and IL-18 in human blood. *J. Immunol.,* **165,** 4615–4623.

ROMANOVSKY, A.A. (2004). Signaling the brain in the early sickness syndrome: are sensory nerves involved? *Front. Biosci.,* **9,** 494–504.

ROTH, J. & DE SOUZA, G.E. (2001). Fever induction pathways: evidence from responses to systemic or local cytokine formation. *Braz. J. Med. Biol. Res.,* **34,** 301–314.

SIKORA, A., LIU, J., BROSNAN, C., BUELL, G., CHESSEL, I. & BLOOM, B.R. (1999). Cutting edge: purinergic signaling regulates radical-mediated bacterial killing mechanisms in macrophages through a $P2X_7$-independent mechanism. *J. Immunol.,* **163,** 558–561.

SOLLE, M., LABASI, J., PERREGAUX, D.G., STAM, E., PETRUSHOVA, N., KOLLER, B.H., GRIFFITHS, R.J. & GABEL, C.A. (2001). Altered cytokine production in mice lacking $P2X_7$ receptors. *J. Biol. Chem.,* **276,** 125–132.

SURPRENANT, A., RASSENDREN, F., KAWASHIMA, E., NORTH, R.A. & BUELL, G. (1996). The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). *Science,* **272,** 735–738.

SUZUKI, T., HIDE, I., IDO, K., KOHSAKA, S., INOUE, K. & NAKATA, Y. (2004). Production and release of neuroprotective tumor necrosis factor by $P2X_7$ receptor-activated microglia. *J. Neurosci.,* **24,** 1–7.

WAAGE, A. (1987). Production and clearance of tumor necrosis factor in rats exposed to endotoxin and dexamethasone. *Clin. Immunol. Immunopathol.,* **45,** 348–355.

WILEY, J.S., CHEN, R. & JAMIESON, G.P. (1993). The ATP^{4-} receptor-operated channel (P2Z class) of human lymphocytes allows Ba^{2+} and $ethidium^{+}$ uptake: Inhibition of fluxes by suramin. *Arch. Biochem. Biophys.,* **305,** 54–60.

(Received March 22, 2005
Accepted April 22, 2005
Published online 20 June 2005)