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Dose-dependent attenuation of lipopolysaccharide-fever by inhibitors of inducible nitric oxide-synthase in guinea pigs

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Abstract

Different doses of aminoguanidine or *S*-methylisothiourea, both predominantly inhibitors of the inducible form of nitric oxide (NO)-synthase, were injected into the arterial circulation of guinea pigs alone or along with 10 μg/kg bacterial lipopolysaccharide. Doses of 10 mg/kg, 50 mg/kg or 250 mg/kg aminoguanidine per se had no influence on abdominal temperature of guinea pigs. Only the highest dose of aminoguanidine (250 mg/kg) completely suppressed the first phase of the biphasic febrile response to lipopolysaccharide-injections. Lipopolysaccharide-fever was not modulated by administration of 10 mg/kg or 50 mg/kg aminoguanidine, when compared to fever in response to injections of lipopolysaccharide along with solvent. Doses of 10 mg/kg or 50 mg/kg *S*-methylisothiourea did not alter abdominal temperature while a dose of 250 mg/kg *S*-methylisothiourea had a lethal effect in guinea pigs. The febrile response to lipopolysaccharide was unimpaired by administration of 10 mg/kg *S*-methylisothiourea, while a dose of 50 mg/kg again attenuated fever predominantly by a suppression of the first fever phase. None of the applied doses of aminoguanidine or *S*-methylisothiourea resulted in a significant attenuation of the lipopolysaccharide-induced circulating cytokines tumor necrosis factor-α (TNF-α) and interleukin-6. The drugs themselves, without lipopolysaccharide-injections, did not enhance or reduce circulating levels of the investigated cytokines. The results indicate that endogenous NO may participate in the induction of lipopolysaccharide-fever and that fever suppression by systemic administration of NO-synthase inhibitors occurs independently from the lipopolysaccharide-induced circulating cytokines.

Keywords: Cytokine; TNF-α (Tumor necrosis factor-α); Interleukin-6; Nitric oxide (NO); Temperature regulation; Radiotelemetry

1. Introduction

Microbial products such as lipopolysaccharide from gram-negative bacteria exert their biological effects in a host organism by the induction of a cytokine cascade (Dinarello, 1991). The cytokines themselves induce a number of enzymes including the inducible form of nitric oxide (NO)-synthase (iNOS). Therefore, the question arises if well-known physiological responses which accompany the activation of the cytokine cascade are, in part, mediated by the cytokine-induced enzyme iNOS. One of the most common responses to infection or inflammation is the development of a febrile response and there is agreement that representative members of the cytokine cascade are involved in the generation of fever (Kluger, 1991; Kozak et al., 1995; Chai et al., 1996; Rothwell, 1997), although especially the early phase of fever is possibly induced without direct participation of cytokines (Blatteis and Sehic, 1997).

Does NO, an important signal created by cytokines, play a role in the manifestation of the febrile response? Recent studies with the aim to obtain an answer to this question were leading, in part, to conflicting results. Some experiments in rabbits suggest that NO might have antipyretic properties (Gourine, 1995; Weihrauch and Riedel, 1997). In cats, NO seems to have no effect on fever at all (Redford et al., 1995). Other studies performed with rats

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(Reimers et al., 1994; Scammell et al., 1996; Roth et al., 1998b), pigs (Parrott et al., 1998) or guinea pigs (Roth et al., 1998c) provide evidence that NO participates in the development of a febrile response. The suggestion for a

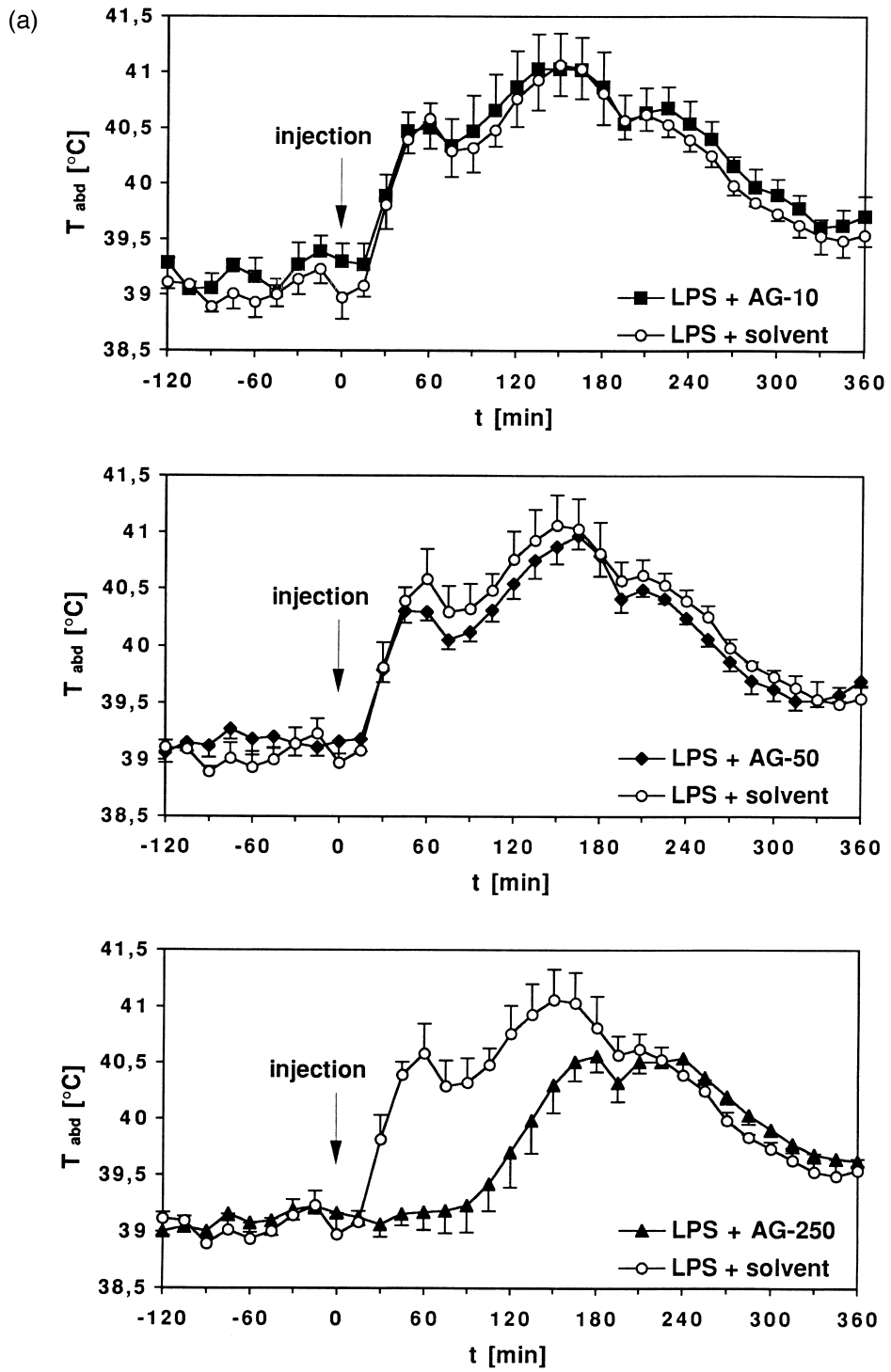

Fig. 1. (a) Febrile responses of four groups of guinea pigs to intra-arterial injections of 10 μg/kg lipopolysaccharide along with 0.9% NaCl (solvent, $N = 6$, open symbols, shown in the upper, middle and lower panel), 10 μg/kg lipopolysaccharide along with 10 mg/kg aminoguanidine ($N = 6$, black symbols in the upper panel), 10 μg/kg lipopolysaccharide along with 50 mg/kg aminoguanidine ($N = 5$, black symbols in the middle panel) or 10 μg/kg lipopolysaccharide along with 250 mg/kg aminoguanidine ($N = 6$, black symbols in the lower panel). Values are presented as means, bars indicate S.E. (b) Abdominal temperature responses of four groups of guinea pigs to intra-arterial injections of 0.9% NaCl (solvent, $N = 5$), 10 mg/kg aminoguanidine ($N = 4$), both shown on the upper panel, 50 mg/kg aminoguanidine ($N = 4$) or 250 mg/kg aminoguanidine ($N = 5$), both shown on the lower panel.

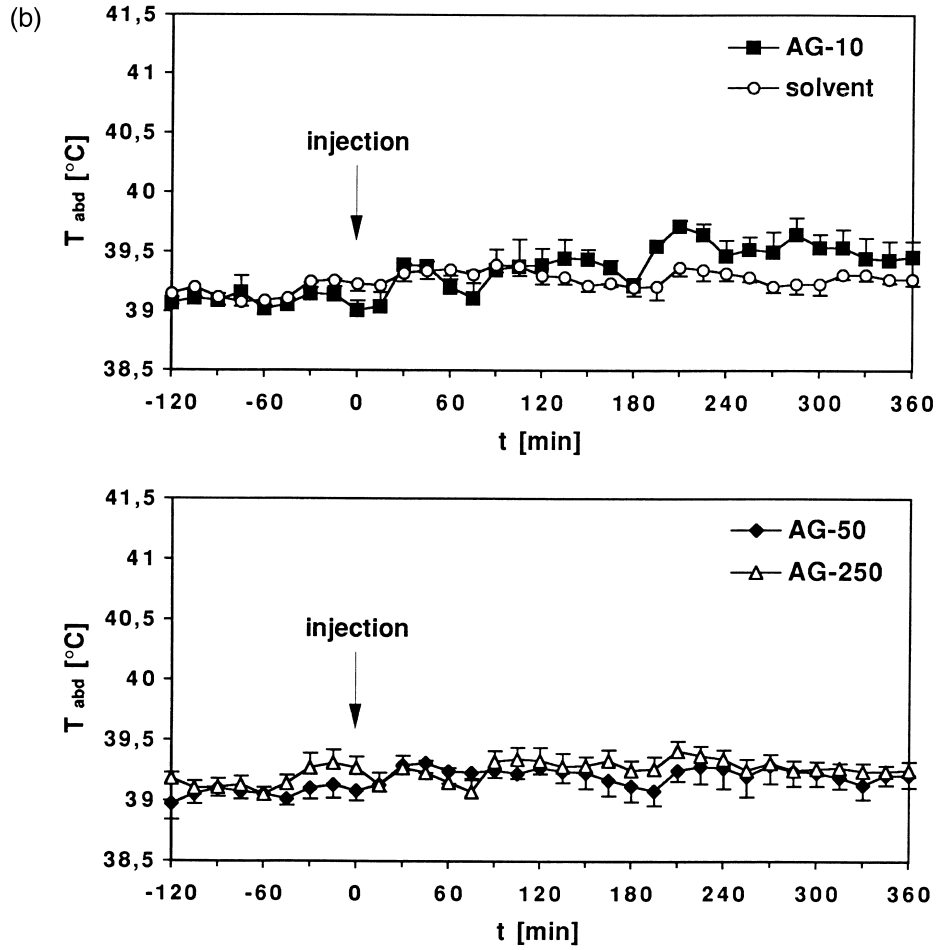

Fig. 1 (*continued*).

propyretic role for NO is predominantly based on the effects of systemic administrations of *N*-nitro-L-arginine-methylester (L-NAME) along with pyrogens, a substance which is regarded as a rather unspecific inhibitor of all isoforms of NO-synthases. Injections of L-NAME directly into the circulation result in attenuation of fever, but this drug per se also induces hypothermia when administered at higher doses (Scammell et al., 1996; Roth et al., 1998c). Although the results of the studies in rats, pigs and guinea pigs suggest that NO might play a role in the generation and maintenance of fever in these species of experimental animals, some questions still remain open: (A) Is the hypothermia which is observed after administration of L-NAME at higher doses due to inhibition of NO-synthesis or is it a drug-effect? (B) Do specific inhibitors of iNOS, the only form of this enzyme which is induced by lipopolysaccharide or cytokines, influence fever in a similar way as the unspecific inhibitor L-NAME? (C) Is there a positive or negative feedback of iNOS inhibitors on the lipopolysaccharide-induced cytokine network which could be responsible for possible modulations of the febrile response? Therefore, we injected different doses of aminoguanidine or *S*-methylisothiourea, both predominantly inhibitors of iNOS (Szabo et al., 1994; Zhao et al., 1996), into the arterial circulation of guinea pigs alone or along with lipopolysaccharide and investigated their influence on body temperature and on circulating cytokines.

2. Materials and methods

2.1. Animals

This study was performed in male guinea pigs with a body mass in the range of 385–410 g at the time of surgery. The animals were housed in individual cages at 22°C and a 12:12 h light:dark cycle (light off at 0700 h). The animals had access to food and water ad libitum. Twice a week the reservoires were filled with fresh pelleted food and water and at the same time the cages were changed. At least one week before the experiment was performed, the animals were prepared surgically (see below). Within 3 days before the experiment the animals were habituated once or twice to the blood sampling procedure by withdrawal of a small volume of blood and flushing back the blood plus about 0.1 ml sterile heparinized saline. The national guidelines for experiments

with vertebrate animals have been followed and for the experimental protocols approval by the local ethics committee has been obtained.

2.2. Surgery

At least one week before the start of the experimental procedure, the animals were chronically implanted with intra-arterial catheters. Briefly, the guinea pigs were anesthetized with 100 mg/kg ketamine hydrochloride and 4 mg/kg xylazine. Polyethylene catheters were inserted through the left carotid artery until reaching the aortic arch and fixed in that position by nonsoluble ligatures. The distal ends of the catheters were tunnelled subcutaneously to the interscapular region of the back where they emerged through the skin. After implantation, the catheters were flushed with sterile heparinized saline and sealed by heating.

2.3. Blood sampling

During the experiment, single blood samples (0.6 ml) were slowly (within 1 min) withdrawn into a sterile heparinized syringe, transferred into a polypropylene tube and immediately centrifuged. A volume of about 0.2 ml sterile 0.9% saline was injected slowly into the catheter after each blood sampling procedure. The blood plasma was stored at −70°C for later determination of cytokines.

2.4. Substances

Bacterial lipopolysaccharide (lipopolysaccharide derived from *Escherichia coli*, O111:B4, Sigma, St. Louis, MO, USA) was dissolved in sterile pyrogen-free 0.9% saline. A dose of 10 μg/kg lipopolysaccharide was used for injections into the intra-arterial catheter.

Aminoguanadine and *S*-methylisothiourea (both obtained from Sigma) were dissolved in sterile pyrogen-free 0.9% saline and injected into the intra-arterial catheters at doses of 10 mg/kg, 50 mg/kg or 250 mg/kg alone or along with lipopolysaccharide. Because of lethal effects of 250 mg/kg *S*-methylisothiourea in three out of four guinea pigs, this dose of *S*-methylisothiourea was not injected along with lipopolysaccharide. Control groups of animals received an equivalent volume of solvent (0.9% sterile pyrogen-free saline) alone or along with lipopolysaccharide.

2.5. Measurement of body temperature

Abdominal temperature was measured by use of battery-operated biotelemetry transmitters (VM-FH-discs, Mini-Mitter, Sunriver, OR, USA) implanted intraperitoneally after placement of the intra-arterial catheter. Output (frequency in Hz) was monitored by an antenna placed under each animal's cage (RA 1000 radioreceivers, Mini-Mitter) and multiplexed by means of a BCM 100 consolidation matrix to an IBM personal computer system. A Dataquest IV data acquisition system (Data Sciences, St. Paul, MN, USA) was used for automatic control of data collection and analysis. Body temperature was monitored and recorded at 5-min intervals. For the analysis and graphical documentation, temperature data of 15-min intervals were used.

2.6. Bioassays for TNF and interleukin-6

Determination of TNF was performed by a bioassay based on the cytotoxic effect of TNF on the mouse fibrosarcoma cell line WEHI 164 subclone 13 (Espevic and Nissen-Meyer, 1986).The assay was performed in sterile, 96-well microtiter plates. Serial dilutions of biological

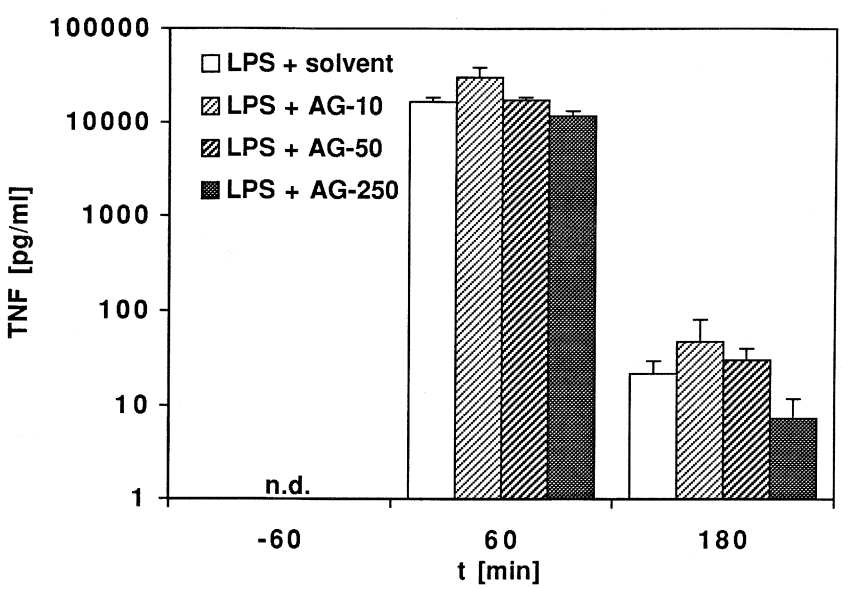

Fig. 2. Lipopolysaccharide-induced levels of bioactive TNF in plasma in the four groups of guinea pigs which were introduced on Fig. 1a. Blood samples were collected at the indicated time intervals during the experiments shown on Fig. 1a. Columns represent means, bars indicate S.E.

samples or different concentrations of TNF-standard (code 88/532, National Institute for Biological Standards and Control, South Mimms, UK) were incubated for 24 h in wells that had been seeded with 50,000 actinomycin D-treated WEHI cells. The number of surviving cells after 24 h was measured by use of the dimethylthiazol-diphenyl tetrazolium bromide (MTT) colorimetric assay (Holt et al., 1991).

Determination of interleukin-6 was performed by a bioassay based on the dose-dependent growth stimulation of interleukin-6 on the B9 hybridoma cell line (Aarden et al., 1987). The assay was performed in sterile, 96-well microtiter plates. In each well, 5000 B9 cells were incubated for 72 h with serial dilutions of biological samples or with different concentrations of interleukin-6 standard (code 89/548, National Institute for Biological Standards and Control). The number of cells in each well was measured by use of the MTT assay (see above).

For both assays, biological samples were adequately prediluted so that serial dilution of samples and standard dilution curves were parallel. The assay detection limits, after allowing for dilutions of samples into the assays, were approximately 3 international units (I.U.)/ml for interleukin-6 and approximately 5 pg/ml for TNF.

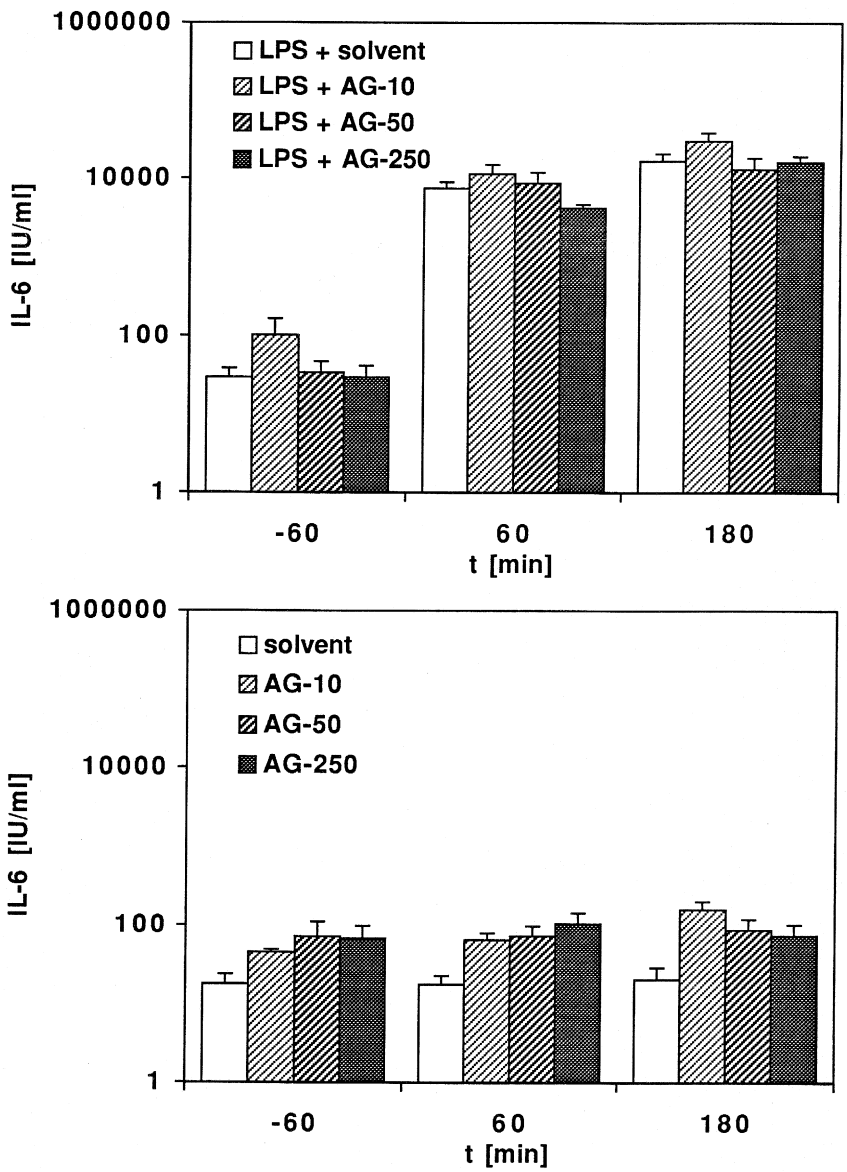

Fig. 3. Upper panel: lipopolysaccharide-induced levels of bioactive interleukin-6 in plasma in the four groups of guinea pigs which were introduced on Fig. 1a. Blood samples were collected during the experiments shown on Fig. 1a. Columns represent means, bars indicate S.E. Lower panel: levels of bioactive interleukin-6 before and after intra-arterial injection of solvent or 10 mg/kg, 50 mg/kg or 250 mg/kg aminoguanidine without coinjection of lipopolysaccharide. The groups of guinea pigs were introduced on Fig. 1b, blood samples were collected at the indicated time intervals during the experiments shown on Fig. 1b. Columns represent means, bars indicate S.E.

2.7. Evaluation and statistics

In graphs of the thermal responses to injections of drugs, the mean changes in abdominal temperature were plotted over time. At each time point abdominal temperatures were expressed as means ± S.E. An analysis of variance (ANOVA) for repeated measures, followed by Scheffe's post hoc test, was used to compare thermal responses. The calculations were carried out on an Apple Macintosh computer using the software package StatView (Abacus Concepts, Berkeley, CA, USA). Circulating levels of TNF and interleukin-6 were compared by Student's *t*-tests. Because the values for cytokine concentrations are not normally distributed, a log-transformation of the cytokine values was performed for the *t*-test.

3. Results

The effects of intra-arterial injections of 10 μg/kg lipopolysaccharide along with solvent or different doses of aminoguanidine (10 mg/kg, 50 mg/kg or 250 mg/kg), or of solvent and the three doses of aminoguanidine alone on abdominal temperature of guinea pigs are summarized in Fig. 1a and b.

Lipopolysaccharide-induced fever was not modulated by administration of 10 mg/kg or 50 mg/kg aminoguanidine. In the group of guinea pigs which received 250 mg/kg aminoguanidine along with lipopolysaccharide, the first part of the febrile response from 30 to 165 min after injection was significantly suppressed ($F = 13.4$; $P = 0.0081$; ANOVA). All tested doses of aminoguanidine per

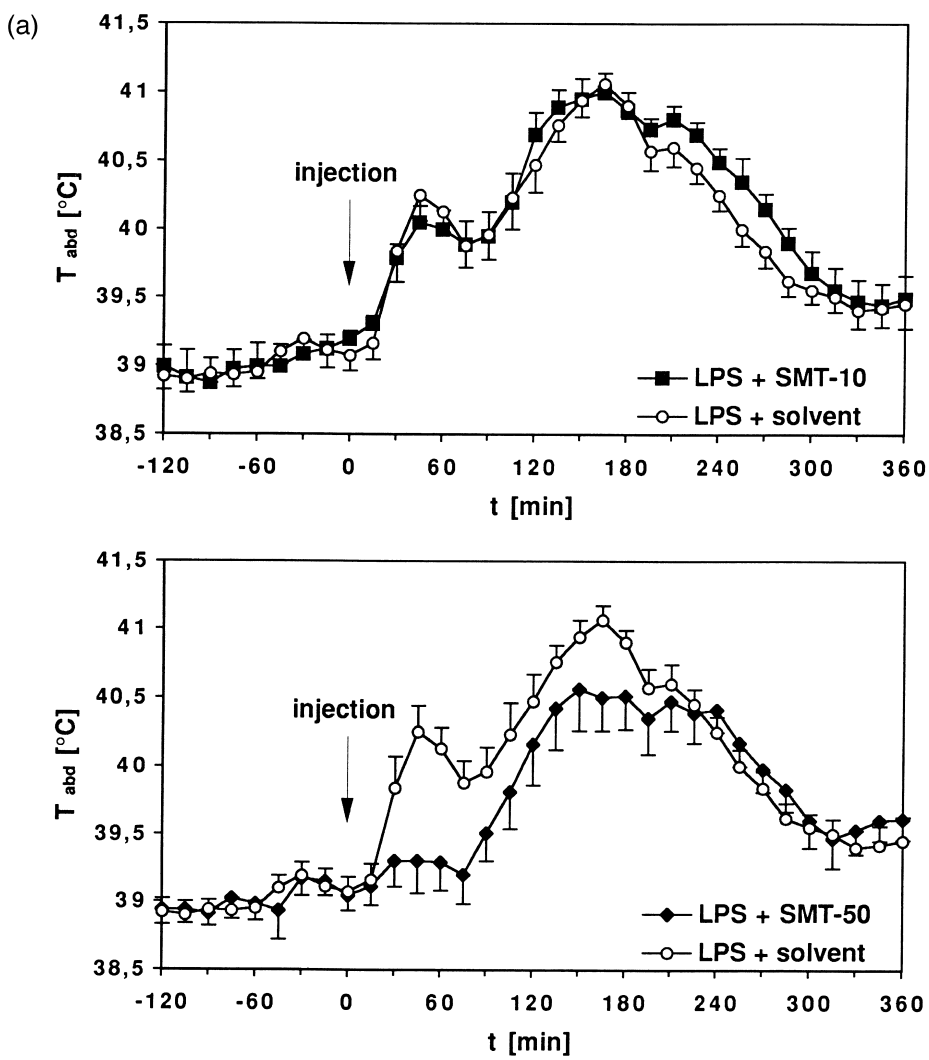

Fig. 4. (a) Febrile responses of three groups of guinea pigs to intra-arterial injections of 10 μg/kg lipopolysaccharide along with 0.9% NaCl (solvent, $N = 7$, open symbols, shown in the upper and lower panel), 10 μg/kg lipopolysaccharide along with 10 mg/kg *S*-methylisothiourea ($N = 6$, black symbols in the upper panel) or 10 μg/kg lipopolysaccharide along with 50 mg/kg *S*-methylisothiourea ($N = 6$, black symbols in the lower panel) Values are presented as means, bars indicate S.E. (b) Abdominal temperature responses of three groups of guinea pigs to intra-arterial injections of 0.9% NaCl (solvent, open symbols, $N = 5$), 10 mg/kg *S*-methylisothiourea ($N = 5$) or 50 mg/kg *S*-methylisothiourea ($N = 5$).

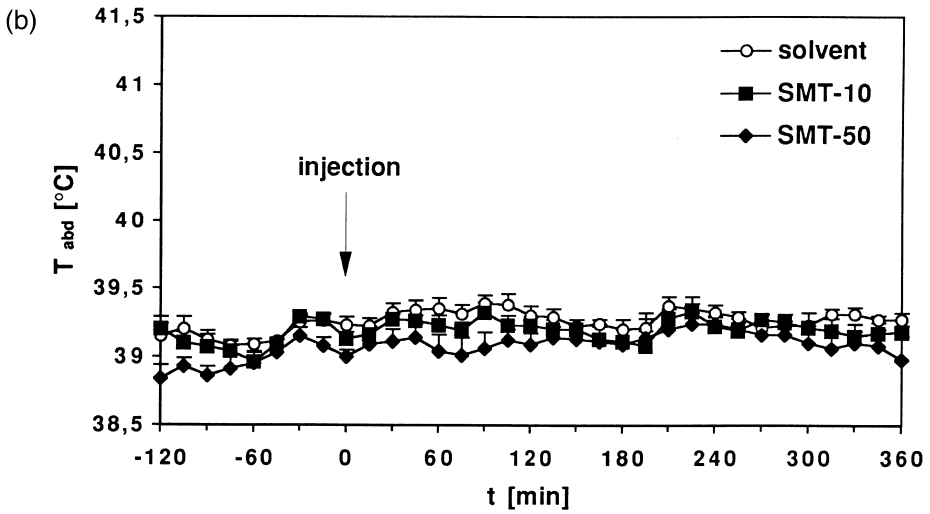

Fig. 4 (*continued*).

se had no apparent influence on body temperature. Figs. 2 and 3 summarize the effects of treatments with solvent or 10 mg/kg, 50 mg/kg and 250 mg/kg aminoguanidine on lipopolysaccharide-induced levels of TNF and interleukin-6 in plasma, or on circulating cytokines without co-injection of lipopolysaccharide.

In summary, lipopolysaccharide induced a strong and rapid rise of circulating TNF in all four groups of guinea pigs within a range of 12,000–30,000 pg/ml. These high levels declined within the next 2 h to values lower than 100 pg/ml in all experimental groups. One hour before administration of drugs no bioactive TNF was detected. One and three hours after injection of solvent or all tested doses of aminoguanidine without lipopolysaccharide, again no TNF was detectable in plasma.

Baseline values of interleukin-6 in the range of 25–100 I.U./ml were measured in all groups of guinea pigs. In response to injections of solvent or of the three tested doses of aminoguanidine alone circulating levels of interleukin-6 remained around the pre-injection values. Administration of lipopolysaccharide along with solvent or 10 mg/kg, 50 mg/kg and 250 mg/kg aminoguanidine induced a rise of interleukin-6 in plasma to several thousand I.U./ml in all experimental groups measured 60 min after injection. Within the following 2 h interleukin-6 in plasma further increased to values between 11,000 and 20,000

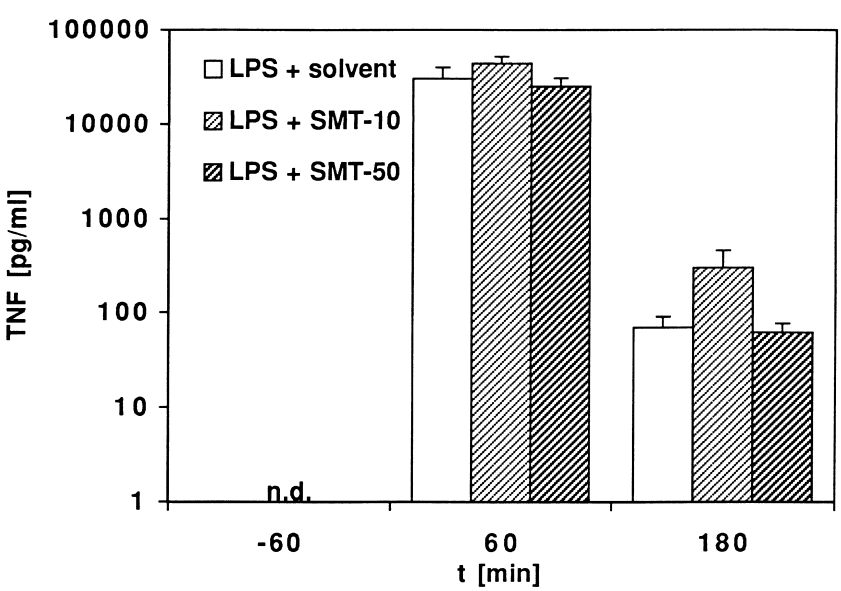

Fig. 5. Lipopolysaccharide-induced levels of bioactive TNF in plasma in the three groups of guinea pigs which were introduced on Fig. 4a. Blood samples were collected at the indicated time intervals during the experiments shown on Fig. 4a. Columns represent means, bars indicate S.E.

I.U./ml. None of the tested doses of aminoguanidine caused a significant alteration of lipopolysaccharide-induced circulating cytokines.

In the next series of experiments, *S*-methylisothiourea, another inhibitor predominantly selective for iNOS was tested for its ability to modulate lipopolysaccharide-fever. The effects on body temperature of intra-arterial injections of solvent, 10 mg/kg or 50 mg/kg *S*-methylisothiourea alone or along with 10 μg/kg lipopolysaccharide are shown on Fig. 4a and b.

The febrile response to lipopolysaccharide was not altered by treatment with 10 mg/kg *S*-methylisothiourea. A dose of 50 mg/kg *S*-methylisothiourea again suppressed the first phase of fever from 30 min to 90 min after injection ($F = 7.2$; $P = 0.0249$; ANOVA). Both doses of *S*-methylisothiourea alone did not cause apparent changes of abdominal temperature. Intra-arterial injections of 250 mg/kg *S*-methylisothiourea had lethal effects in three out of four guinea pigs. We therefore decided not to use higher doses of this drug than 50 mg/kg. Figs. 5 and 6 summarize the effects of *S*-methylisothiourea on circulating TNF and interleukin-6.

The kinetics and quantitative values of lipopolysaccharide-induced levels of TNF and interleukin-6 in plasma

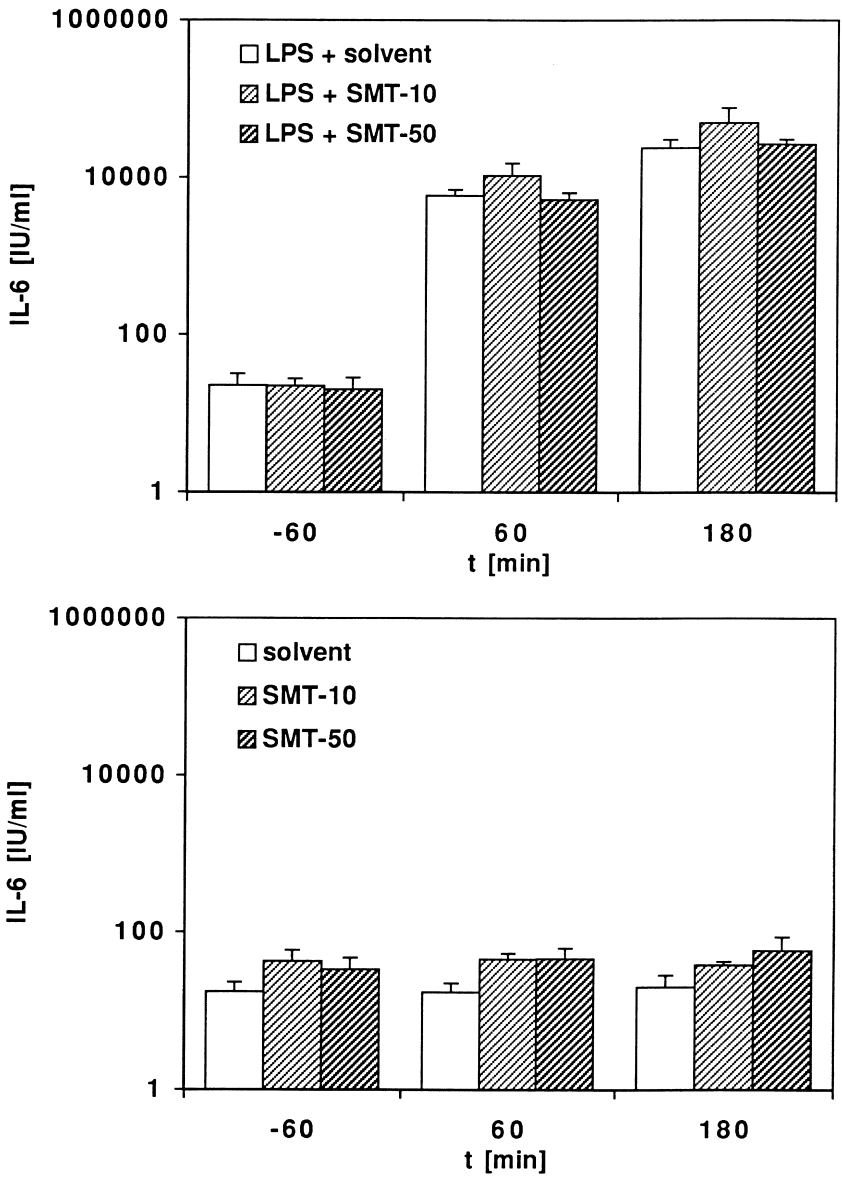

Fig. 6. Upper panel: lipopolysaccharide-induced levels of bioactive interleukin-6 in plasma in the three groups of guinea pigs which were introduced on Fig. 4a. Blood samples were collected at the indicated time intervals during the experiments shown on Fig. 4a. Columns represent means, bars indicate S.E. Lower panel: levels of bioactive interleukin-6 before and after intra-arterial injection of solvent, 10 mg/kg or 50 mg/kg *S*-methylisothiourea without coinjection of lipopolysaccharide. The groups of guinea pigs were introduced on Fig. 4b, blood samples were collected during the experiments shown on Fig. 4b. Columns represent means, bars indicate S.E.

were similar to those which were obtained in the experiments with aminoguanidine, shown on Figs. 2 and 3. Again, administration of 10 mg/kg or 50 mg/kg *S*-methylisothiourea did not alter lipopolysaccharide-induced circulating cytokines. Both doses of *S*-methylisothiourea per se had no influence on levels of bioactive TNF and interleukin-6 in plasma: TNF remained below the limit of detection, interleukin-6 remained around its baseline value.

4. Discussion

The main finding of this study is that the iNOS inhibitors aminoguanidine and *S*-methylisothiourea cause a dose dependent suppression of the first phase of the biphasic lipopolysaccharide-fever in guinea pigs without any influence on lipopolysaccharide-induced amounts of circulating cytokines, putative mediators of the febrile response. Further, in contrast to the unspecific NOS inhibitor L-NAME, both drugs per se did not cause hypothermia. A number of questions may arise concerning this, in part, surprising result. What role can endogenous formation of NO play in the generation of fever? By which mechanisms can iNOS-inhibitors suppress the first phase of lipopolysaccharide-fever? Is the time course of lipopolysaccharide-induced activation of iNOS consistent with our finding?

4.1. The role of NO in fever

The current knowledge about putative functions of NO in temperature regulation and fever has been reviewed recently (Schmid et al., 1998). The collection of data presented in this review indicates some striking species-specific differences with regard to the effects of NO on body temperature control and fever. Clearly, NO can act as a thermolytic substance due to its vasodilative properties and thereby the improved circulatory heat transfer to the skin (Mathai et al., 1995). In rabbits which predominantly use circulatory effector mechanisms to induce a fever, consequently antipyretic properties of NO are observed (Gourine, 1995; Weihrauch and Riedel, 1997). In rats, pigs and guinea pigs suggestions for a propyretic role of NO are based on the observation that administration of the unspecific NOS inhibitor L-NAME results in a suppression of parts of the febrile response (Reimers et al., 1994; Scammell et al., 1996; Parrott et al., 1998; Roth et al., 1998b; Roth et al., 1998c). The results shown on Figs. 1 and 4 of this paper demonstrate dose-dependent antipyretic effects of the iNOS inhibitors aminoguanidine and *S*-methylisothiourea. Due to the fact that constitutive (c)NOS and iNOS are so ubiquitous, actions of NO in the periphery of the body or within the central nervous system may contribute to the attenuation of fever by NOS inhibitors in rats, pigs and guinea pigs. Experiments in rats indicate that NO exerts a thermogenic action in brown adipose tissue since administration of a NOS blocker reduced sympathetic nervous outflow to this tissue and thereby inhibited metabolic heat production (De Luca et al., 1995). In small animals which use activation of brown fat by sympathetic stimulation as an important effector for fever induction by lipopolysaccharide (Jepson et al., 1988), the peripheral contribution of NO in this process can, in part, explain the reduction of fever by administration of NOS inhibitors. An alternative or additional reason for the observed suppression of the early phase of fever could be a rapid activation of endogenous antipyretic pathways by systemic administration of NOS inhibitors. Indeed, intravenous injections of L-NAME, an unspecific NOS inhibitor, augments the increase in circulating levels of adrenocorticotrophic hormone (ACTH) and corticosteroids induced by administration of pyrogenic cytokines (interleukin-1β, interleukin-6, TNF) dramatically (Rivier, 1995; Kim and Rivier, 1998). This effect manifests itself as early as 15–30 min after administration of cytokines along with L-NAME (Kim and Rivier, 1998). The time course of the enhanced activation of the antipyretically acting hypothalamic–pituitary–adrenal-axis is consistent with our observation of a suppression of the first phase of lipopolysaccharide-fever. However, we applied inhibitors of iNOS in our experiments and therefore the question arises, how rapidly iNOS is induced after administration of lipopolysaccharide or cytokines.

4.2. The role of iNOS in lipopolysaccharide-induced responses

Resting unstimulated cells do not express iNOS, but after stimulation with lipopolysaccharide or cytokines immune cells such as macrophages and a variety of cell types outside the classical immune system can express iNOS (Morris and Billiar, 1994). The formation of NO by iNOS is thus delayed until the enzyme is synthetized. Clearly, a number of lipopolysaccharide-induced effects can be blocked or attenuated by ''anti-iNOS-strategies''. Treatment of mice with a selective inhibitor of iNOS reduces liver damage and mortality in response to challenge with a lethal dose of lipopolysaccharide (Liaudet et al., 1998). In knockout mice, deficient in iNOS, the fall in central arterial pressure and mortality after injection of a septic shock inducing dose of lipopolysaccharide are reduced in comparison to the responses of wild-type mice (MacMicking et al., 1995). If production of NO by iNOS is responsible for the suppression of the first phase of lipopolysaccharide-fever, very rapid induction of this enzyme would be a prerequisite. Some studies were performed with the aim to demonstrate iNOS protein in the brain in response to a fever-inducing (not a septic or even lethal) dose of lipopolysaccharide. Only a small population of iNOS positive microglial cells appearing several hours after administration of lipopolysaccharide can be documented in rats (Van Dam et al., 1995). No significant elevation of NOS activity

is detected in brain structures relevant for fever induction in guinea pigs a short time after lipopolysaccharide-injection (Sehic et al., 1997). Moderate presence of iNOS can be detected in the hypothalamus of rats some hours after administration of lipopolysaccharide (Minano et al., 1997). Finally, a recent study to obtain information about the time course of expression of all isoforms of NOS in the brain after lipopolysaccharide-challenge indicates that rather an upregulation of both constitutive forms of NOS accompanies lipopolysaccharide-fever while expression of iNOS occurs only in response to high (septic) doses of lipopolysaccharide (Gath et al., 1999). Taking together all these findings, it seems rather unlikely that the time course of iNOS induction by lipopolysaccharide is consistent with the suppression of the first fever phase which we observed after treatment with iNOS inhibitors. We rather believe that the continuously present cNOS isoforms are also inhibited by the highest doses of aminoguanidine and *S*-methylisothiourea which are predominantly inhibitors of iNOS. If that is true, there remains a possible role for NO in the generation of the first phase of lipopolysaccharide-induced fever.

4.3. Biphasic fever: which signal triggers the first phase?

Some years ago, it has been pointed out correctly that various experimental procedures which attenuate fever usually cause an inhibition of the second phase only or of both febrile phases together (Romanowsky and Blatteis, 1995). Some of our own recent experiments support this statement. For example, when we completely neutralize lipopolysaccharide-induced circulating TNF by its soluble receptor, we observe a significant reduction of interleukin-6, a pyrogenic mediator downstream in the cytokine cascade, and an attenuation of only the second phase of lipopolysaccharide-fever (Roth et al., 1998a). The first fever phase seems thus to develop independently from the presence or absence of circulating cytokines. It has been suggested that the quick onset of lipopolysaccharide-fever after intravenous or intra-arterial injection of this pyrogen is triggered by a very rapidly evoked neuroactive substance which may activate a nerval signal transfer to the brain (Blatteis and Sehic, 1997). Alternatively, lipopolysaccharide per se might directly activate the first wave of fever by its interaction with a receptor involved in the fever pathway. An exciting hypothesis was introduced recently which would be compatible with the quick onset of lipopolysaccharide-induced fever prior to the formation of putative endogenous mediators (Dinarello et al., 1999). An ancient receptor protein, a so termed ‘‘drosophila toll protein’’, which belongs to the interleukin-1 receptor family, can obviously be activated by lipopolysaccharide and by interleukin-1 (Dinarello et al., 1999). The first phase of fever could thus directly be induced by lipopolysaccharide via this ancient toll receptor. The subsequent production of interleukin-1 and other pyrogenic cytokines could predominantly be responsible for the second phase of fever. We are unable to decide at present if the iNOS inhibiting drugs aminoguanidine and *S*-methylisothiourea interfere in the pathway of activation of the ancient toll receptor which belongs to the interleukin-1 receptor family. The fact that interleukin-1-induced fever can be suppressed by NOS inhibitors even more effectively than lipopolysaccharide-induced fever (Roth et al., 1998b) raises the possibility that some NOS inhibitors are indeed interfering with this receptor. This interesting question merits further studies on the role of the ancient interleukin-1 toll receptor in lipopolysaccharide-induced fever and its possible interaction with NOS-inhibitors.

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