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European Journal of Pharmacology 467 (2003) 133–140
www.elsevier.com/locate/ejphar

Fever induced by platelet-derived growth factor, in contrast to fever induced by lipopolysaccharide, depends only on nitric oxide, but not on carbon monoxide pathway

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Received 28 November 2002; received in revised form 14 March 2003; accepted 18 March 2003

Abstract

Platelet-derived growth factor (PDGF) is a multifunctional protein which is known to induce a febrile response when injected intracerebroventricularly. The gaseous neurotransmitters, nitric oxide (NO) and carbon monoxide (CO), are both known to exert thermoregulatory effects and to participate in lipopolysaccharide-induced fever. In this study, we investigated the role of NO and CO in the febrile response to PDGF-BB in rats. Intracerebroventricular (i.c.v.) injection of PDGF-BB produced a dose-dependent increase in body temperature. This increase in body temperature induced by PDGF-BB was exacerbated by N^G-nitro-L-arginine methyl ester (L-NAME—a nonselective NO synthase inhibitor) and *S*-methyl-L-thiocitrulline treatment [SMTC—a neuronal NOS (nNOS) selective inhibitor], but not by aminoguanidine treatment [an inducible NOS (iNOS) selective inhibitor]. Zinc deuteroporphyrin 2,4-bis glycol treatment (ZnDPBG—a nonselective heme oxygenase (HO) blocker) did not affect PDGF-BB fever. Our data indicate that the NO but not the CO pathway participates in PDGF-BB fever. Furthermore, our data show that nNOS is the NOS isoform responsible for NO synthesis in this response.

Keywords: Nitric oxide (NO) synthase; Heme oxygenase; Fever; PDGF (platelet-derived growth factor)

1. Introduction

Fever is a regulated elevation of body temperature characteristically exhibited by most species in response to invasion by infectious agents. It is the most important nonspecific systemic reaction designed to combat the deleterious effects of invading pathogens to restore health to the afflicted host (Kluger, 1991). Fever is an integrated response of the body, involving the release of endogenous pyrogens (interleukin-1, interleukin-6, tumor necrosis factor-α, etc.) by immune cells (Kluger, 1991), the transfer of these immune signals to the brain (Blatteis et al., 1998) and the coordinated response of several brain regions to increase the thermoregulatory set point and consequently body temperature. In the brain, an overproduction of prostaglandin E_2 is thought to be a critical step for the induction of fever, with the preoptic region of the anterior hypothalamus being the presumed site of action (Blatteis et al., 1998).

Platelet-derived growth factor (PDGF) is a multifunctional protein that exerts important actions in the central nervous system (CNS—Valenzuela et al., 1997). The three different isoforms of PDGF (PDGF-AA, PDGF-AB and PDGF-BB) act by binding to PDGF receptors with intrinsic tyrosine kinase activity (Heldin and Westermark, 1999). Both PDGF and its receptors are widely expressed in neurons and glial cells throughout the CNS, including the preoptic region of the anterior hypothalamus and other regions important for the control of body temperature (Valenzuela et al., 1997). Recently, it was demonstrated that PDGF acts as an endogenous CNS mediator of the febrile response in rats (Pela et al., 1998, 2000).

Nitric oxide (NO), a diffusible free radical gas, has been shown to have signaling properties (Dawson and Snyder, 1994; Moncada et al., 1991), many of which are the result of

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doi:10.1016/S0014-2999(03)01636-4

activation of soluble guanylate cyclase and the consequent increase in intracellular cyclic GMP levels (Dawson and Snyder, 1994; Moncada et al., 1991). Endogenous NO production is catalyzed by the enzyme NO synthase (NOS) during the conversion of L-arginine to L-citrulline. Three NOS isoforms have been identified, among them neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutively expressed, whereas inducible NOS (iNOS) is usually not expressed under basal conditions but may be overexpressed in response to a series of stimuli (Dawson and Snyder, 1994; Moncada et al., 1991).

Since the beginning of the 1990s, a growing body of evidence has given support to the physiological actions of another gaseous compound, carbon monoxide (CO), which has been shown to be a vasoactive substance and to act as a neurotransmitter or neuromodulator (for reviews, see Dawson and Snyder, 1994; Johnson et al., 1997). Endogenously, heme oxygenase (HO) catalyzes the rate-limiting step in the oxidative degradation of heme to biliverdine, free iron and CO (Dawson and Snyder, 1994; Johnson et al., 1997). At least three distinct HO isoforms have been identified: HO-1, HO-2 and HO-3 (Maines, 1997). HO-2 is constitutively expressed throughout the body, including the CNS (Marks et al., 1991), whereas HO-1 is absent or expressed at low levels in tissues, but can be overexpressed in response to a series of stimuli (Dawson and Snyder, 1994; Johnson et al., 1997; Maines, 1997). Very little is known about the heme oxygenase 3 isoform (Dawson and Snyder, 1994; Johnson et al., 1997).

Over the years, evidence has been growing in support of the importance of NO and CO as neuromodulators involved in the control of body temperature and fever. Both NO and CO have been shown to participate in lipopolysaccharide-induced fever (Steiner and Branco, 2000, 2001b). However, the involvement of NO and CO in PDGF-induced fever has not yet been assessed. Thus, the aim of the present study was to investigate the role of NO and HO/CO pathways in PDGF-induced fever by using the nonselective NOS inhibitor, N^G-nitro-L-arginine-methyl-ester (L-NAME) and the nonselective HO blocker, zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG). We also identified the NOS isoform involved in this response by using the more selective nNOS inhibitor, *S*-methyl-thiocitrulline (SMTC), and the selective iNOS inhibitor, aminoguanidine.

2. Materials and methods

2.1. Animals

Experiments were performed on adult male Wistar rats weighing 220–320 g, housed at controlled temperature (24 ± 1 °C) and exposed to a daily 12:12 light–dark cycle. The animals were allowed free access to water and food. Experiments started at 9:00 a.m. Animal care was provided in compliance with the guidelines set by the American Physiological Society (2002).

2.2. Drugs

Endotoxin-free platelet-derived growth factor (PDGF-BB), N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME), *S*-methyl- L-thiocitrulline (SMTC) and aminoguanidine were purchased from Sigma (USA). These drugs were dissolved in pyrogen-free sterile saline. Zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) was purchased from Porphyrin Products (USA) and dissolved in 50 mM Na_2CO_3.

2.3. Surgery

Rats were anesthetized with an intraperitoneal injection of 250 mg/kg (1 ml/100 g) of 2,2,2-tribromoethanol and fixed to a stereotaxic frame. A stainless steel guide cannula (0.7 mm OD) was introduced into the right lateral cerebral ventricle (coordinates: A: −1.0 mm, L: −1.6 mm, D: 3.2–3.7 mm) (Paxinos and Watson, 1998). The displacement of the meniscus in a water manometer ensured correct positioning of the cannula in the lateral ventricle. The cannula was attached to the bone with stainless steel screws and acrylic cement. A tight-fitting stylet was kept inside the guide cannula to prevent occlusion.

The insertion of a biotelemetry probe capsule (model: ER-4000; Mini-Mitter, Sunriver, OR) into the peritoneal cavity was accomplished via a paramedian laparotomy. The wound was then closed with skin sutures and the implanted capsule was used for measurements of body temperature. Some animals were also implanted with a silastic catheter through the external jugular vein. The surgical procedures were performed over a period of ∼20 min. After surgery, the animals were treated with 1,200,000 units of antibiotic and allowed to recover for 5 days. During this period, the catheters were flushed daily with heparinized saline.

2.4. Body temperature measurements

For all protocols, body temperature was measured by biotelemetry (Mini-Mitter, USA) at 5 min and plotted at 10-min intervals, during a period of 60 min before and 360 min after the treatments. Before each treatment, initial body temperature was determined as the average of the last five body temperature measurements made at 5-min intervals. The rats were left undisturbed for at least 24 h before the experiment.

2.5. Experimental protocol

2.5.1. Determination of the effect of the PDGF-BB on body temperature

A 705-LT, 10-μl Hamilton syringe and a dental injection needle (200 μm OD; Missy) were used for intracerebroventricular (i.c.v.) injections. Injection was performed over a period of 2 min, and 1 min was allowed to elapse before the

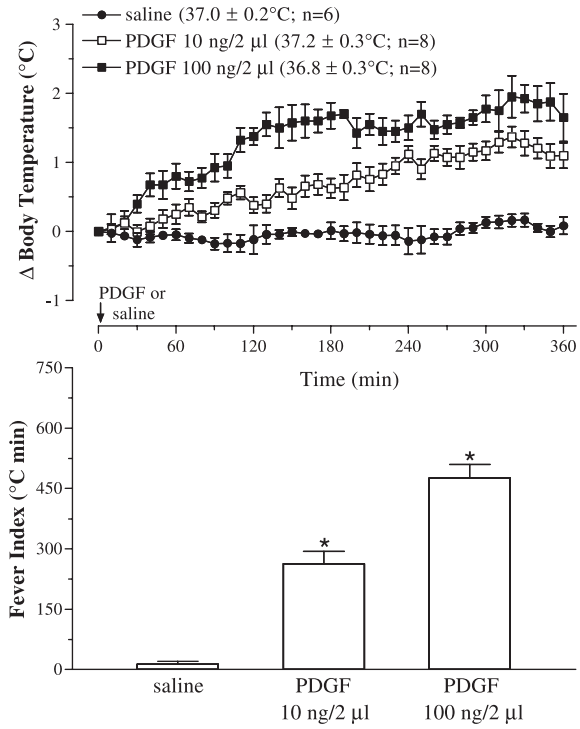

Fig. 1. Top: time course of increase in body temperature induced by intracerebroventricular (i.c.v.) injection of PDGF-BB. The numbers in parentheses are related to initial body temperature (the average of the last five measurements made at 5-min intervals before all treatments). Bottom: fever index after saline or PDGF (10 or 100 ng) i.c.v. injection. Values are means ± S.E.M. *$P<0.05$ vs. saline.

injection needle was removed from the guide cannula to avoid reflux.

Rats received an i.c.v. injection of PDGF-BB (10 and 100 ng/2 µl) or saline. Body temperature was then monitored over a period of 360 min. Doses were chosen on the basis of pilot experiments and of previous studies (Pela et al., 1998, 2000).

2.5.2. Determination of the effect of nonselective NOS blocker, L-NAME on PDGF-BB-induced fever

The animals received an i.c.v injection of L-NAME (200 µg/1 µl) or D-NAME followed by an i.c.v. injection of saline or PDGF-BB (10 ng/2 µl) 30 min later. To check if the L-NAME effect is centrally mediated and not due to a systemic action of the drug, another group of animals received an intravenous (i.v.) injection of L-NAME or D-NAME at the same dose as used intracerebroventricularly, 30 min before an i.c.v. injection of PDGF-BB. Body temperature was monitored as described earlier. The L-NAME dose was chosen on the basis of a previous study from our laboratory (Almeida et al., 1999).

2.5.3. Determination of the NOS isoform involved in PDGF-BB-induced fever

The rats received an i.c.v injection of the selective iNOS blocker aminoguanidine (250 µg/kg), or the selective nNOS blocker, SMTC (2.5 µmol/1 µl), 30 min before an i.c.v. injection of PDGF-BB (10 ng/2 µl). Body temperature was monitored as described before. Control rats received an i.c.v. injection of saline in both cases. To verify if the SMTC effect is centrally mediated and not due to a systemic action of the drug, another group of animals received an intravenous injection of SMTC at the same dose as used intracerebroventricularly, 30 min before an i.c.v. injection of PDGF-BB. Doses were chosen on the basis of pilot experiments.

2.5.4. Determination of the effect of the nonselective HO blocker, ZnDPBG, on PDGF-BB-induced fever

Animals received an i.c.v. injection of ZnDPBG (200 nmol/4 µl) or its vehicle (50 mM Na_2CO_3) followed by an i.c.v. injection of saline or PDGF-BB (10 ng/2 µl) 30 min later. Another group of animals received an i.c.v. injection of ZnDPBG or its vehicle 120 min after an i.c.v. injection of PDGF-BB. This period of time was chosen on the basis of the time course of the response of PDGF-BB-induced fever. Body temperature was monitored as described earlier. The ZnDPBG dose was chosen on the basis of previous studies from our laboratory (Almeida and Branco, 2002; Steiner and Branco, 2001a).

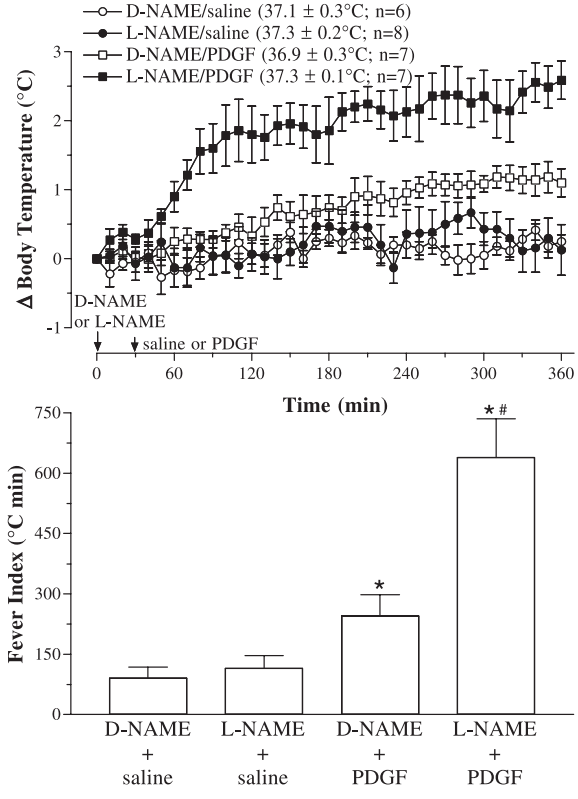

Fig. 2. Top: time course of the effect of L-NAME on PDGF-BB-induced fever. The numbers in parentheses are related to initial body temperature (the average of the last five measurements made at 5-min intervals before all treatments). Bottom: fever index after L-NAME or D-NAME followed by i.c.v. injection of PDGF-BB or saline. Values are means ± S.E.M. *$P<0.05$ vs. D-NAME + saline; $^{\#}P<0.05$ vs. D-NAME + PDGF (10 ng).

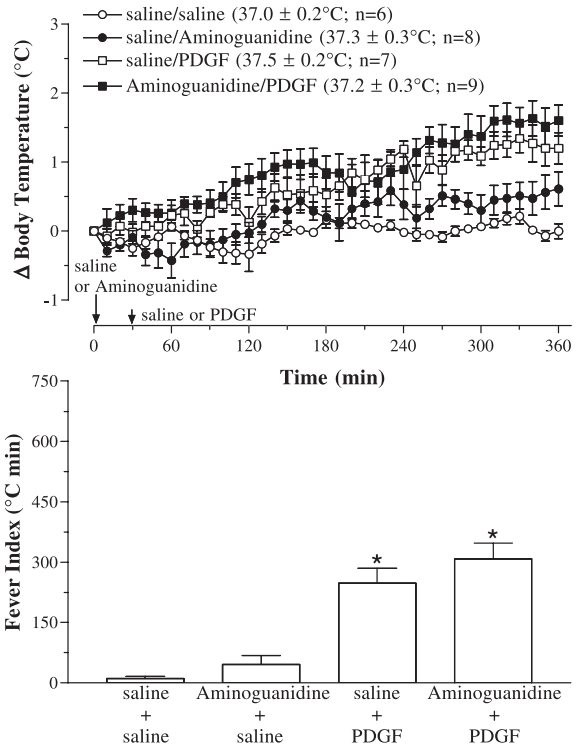

Fig. 3. Top: effect of aminoguanidine on PDGF-BB-induced fever. The numbers in parentheses are related to initial body temperature (the average of the last five measurements made at 5-min intervals before all treatments). Bottom: fever index after saline or aminoguanidine followed by i.c.v. injection of PDGF-BB or saline. Values are means ± S.E.M. *$P<0.05$ vs. saline + saline.

2.6. Statistical analysis

All values are reported as means ± S.E.M. The values for body temperature are the changes from the basal values (the average of the last five body temperature measurements made at 10-min intervals before all treatments). Two-way analysis of variance (ANOVA) was used for data analyses, followed by a point-by-point ANOVA and by the Tukey–Kramer multiple comparisons test to assess the significance of differences between groups. Fever indexes (°C min) were used to improve the data analysis and were calculated as areas under the body temperature curves. Statistical analyses were performed on these data using ANOVA followed by the Tukey–Kramer multiple comparisons test. Values of $P<0.05$ were considered significant.

3. Results

Injection of PDGF-BB into the lateral ventricle induced a dose-dependent increase in body temperature (Fig. 1). Injection of 10 and 100 ng of PDGF-BB induced a statistically significant increase in body temperature from 70 to 360 min and from 40 to 360 min after injection, respectively. Fever indexes were 13.3 ± 9.1, 265.6 ± 56.5 and 421.6 ± 92.9 °C min, in animals injected with saline, PDGF-BB 10 or 100 ng, respectively.

Fig. 2 shows the effect of L-NAME on PDGF-BB-induced fever. i.c.v. injection of D-NAME or L-NAME followed by saline injection caused no significant changes in body temperature (fever indexes: 90.4 ± 25.5 and 115.1 ± 31.6 °C min, respectively). i.c.v. injection of D-NAME combined with 10 ng PDGF-BB caused a significant increase in body temperature (fever index: 244.7 ± 52.7 °C min; $P<0.05$ × D-NAME + saline). In animals injected intracerebroventricularly with L-NAME followed by 10 ng of PDGF-BB, a greater increase in body temperature was observed, which was significant from 60 to 360 min when compared to that in animals injected with D-NAME followed by PDGF-BB (fever index: 638.7 ± 90.0 °C min; $P<0.05$ × D-NAME + PDGF). i.v. injection of L-NAME followed by i.c.v. injection of PDGF-BB caused an increase in body temperature similar to that observed when animals were injected intravenously with D-NAME and intracerebroventricularly with PDGF-BB (fever indexes: 247.1 ± 34.2 and 256.2 ± 27.7 °C min, respectively, $P>0.05$).

i.c.v. injection of saline or aminoguanidine (a selective iNOS inhibitor) combined with i.c.v. injection of saline caused no significant changes in body temperature (fever indexes: 10.2 ± 5.4 and 45.7 ± 21.9 °C min, respectively). The PDGF-BB-induced rise in body temperature was sim-

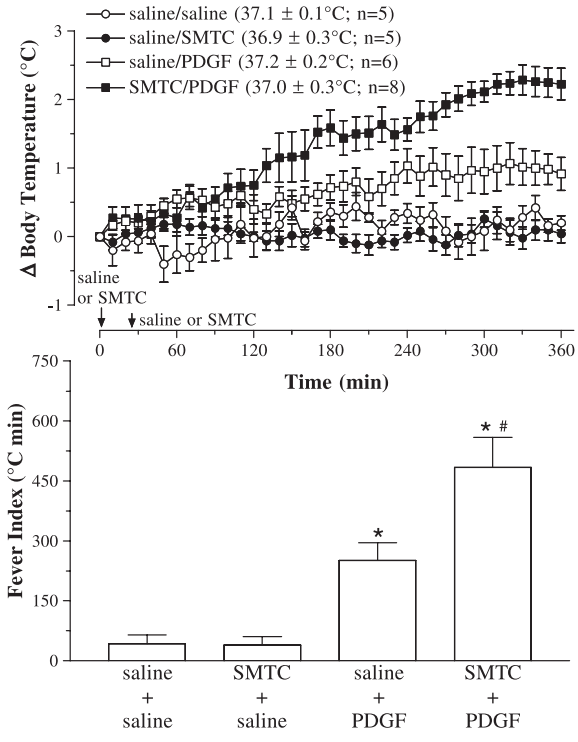

Fig. 4. Top: time course of the effect of SMTC on PDGF-BB-induced fever. The numbers in parentheses are related to initial body temperature (the average of the last five measurements made at 5-min intervals before all treatments). Bottom: fever index after SMTC or saline followed by i.c.v. injection of PDGF-BB or saline. Values are means ± S.E.M. *$P<0.05$ vs. saline + saline; #$P<0.05$ vs. saline + PDGF (10 ng).

ilar in animals injected with saline or aminoguanidine (fever indexes: 247.6 ± 37.4 and 307.9 ± 43.1 °C min, respectively; $P>0.05$). These data are illustrated in Fig. 3.

Fig. 4 shows the effect of SMTC (a selective nNOS blocker) treatment on PDGF-BB-induced fever. No significant change in body temperature was observed after i.c.v. injection of saline or SMTC followed by i.c.v. saline (fever indexes: 42.2 ± 22.3 and 38.8 ± 21.8 °C min, respectively). In animals injected intracerebroventricularly with saline followed by PDGF-BB, a typical rise in body temperature was observed (fever index: 250.8 ± 44.8 °C min). A greater rise in body temperature was observed when the animals were treated with SMTC and PDGF-BB which was significant from 170 to 230 min and from 250 to 360 min when compared to that in animals injected with saline followed by PDGF-BB (fever index: 485.9 ± 74.6 °C min; $P>0.05 \times$ saline + PDGF-BB). i.v. injection of SMTC followed by i.c.v. injection of PDGF-BB caused an increase in body temperature similar to that observed when animals were injected intravenously with saline and intracerebroventricularly with PDGF-BB (fever indexes: 219.8 ± 37.8 and 251.3 ± 21.9 °C min; $P>0.05 \times$ saline + PDGF).

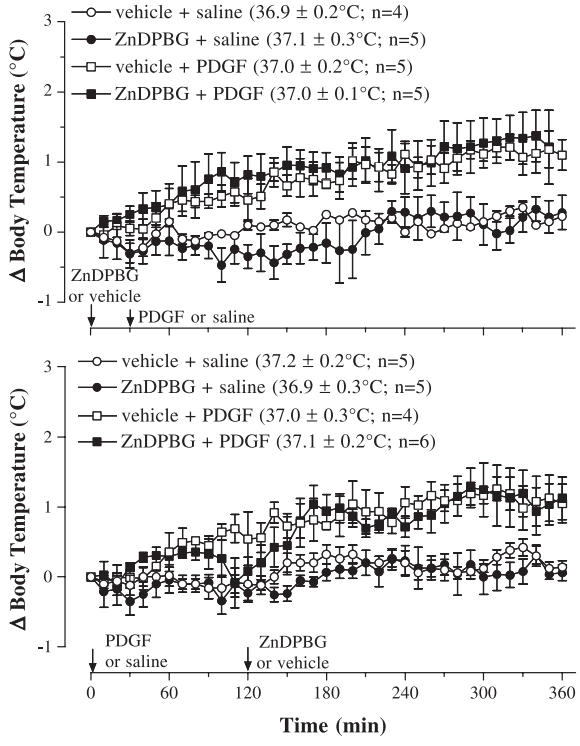

Fig. 5. Time course of the effect of ZnDPBG on PDGF-BB-induced fever. The numbers in parentheses are related to initial body temperature (the average of the last five measurements made at 5-min intervals before all treatments). Top: rats were pretreated with ZnDPBG at time 0 and injected with PDGF-BB at time 30 min. Bottom: rats were injected with PDGF-BB at time 0 and treated with ZnDPBG at time 120 min. Values are means ± S.E.M. There were no significant differences between animals treated with ZnDPBG plus PDGF-BB or with ZnDPBG vehicle (50 mM Na_2CO_3) plus PDGF-BB.

Fig. 5 shows the effect of ZnDPBG on PDGF-BB-induced fever. i.c.v. injection of ZnDPBG or its vehicle (50 mM Na_2CO_3) followed by i.c.v. injection of saline caused no significant changes in body temperature (fever indexes: 40.6 ± 19.3 and 32.2 ± 15.1 °C min, respectively). The rise in body temperature induced by PDGF-BB was similar in animals injected with ZnDPBG or its vehicle (fever indexes: 201.6 ± 63.9 and 265.6 ± 29.2 °C min, respectively). Similar results were found when ZnDPBG or its vehicle was injected 120 min after i.c.v. injection of PDGF-BB: no significant change in body temperature was observed when ZnDPBG or its vehicle was injected in combination with saline (fever indexes: 48.8 ± 24.8 and 26.0 ± 21.1 °C min, respectively) and PDGF-BB-induced fever was similar in animals injected with ZnDPBG or its vehicle (fever indexes: 253.0 ± 47.9 and 275.0 ± 35.7 °C min, respectively).

4. Discussion

The major finding of the present study was that PDGF-BB-induced fever depends only on the NO but not on the HO/CO pathway in the CNS, because injection of the NOS blocker, L-NAME, exacerbated the fever induced by PDGF-BB, whereas ZnDPBG, the HO blocker did not affect PDGF-BB-induced fever. This mechanism differs from that described for lipopolysaccharide-induced fever, where both NO and HO/CO pathways have been shown to be involved. Moreover, we showed that the nNOS blocker, SMTC, but not the iNOS blocker, aminoguanidine, increased PDGF-BB-induced fever, indicating that neuronal NOS is the NOS isoform responsible for NO synthesis in this response.

PDGF is a protein that exerts important actions in different tissues, including the CNS (Valenzuela et al., 1997). Originally, PDGF was identified as a constituent of whole blood serum and was subsequently purified from human platelets. Recent studies have shown that PDGF can be synthesized by a number of different cell types such as macrophages, fibroblasts, vascular smooth muscle cells, neurons and glial cells (for review, see Heldin and Westermark, 1999). PDGF and PDGF receptors are widely expressed in the different regions of the CNS, including the hypothalamus, which is an important site for the control of body temperature and fever (Valenzuela et al., 1997). PDGF synthesis is often increased in response to external stimuli, such as exposure to low oxygen tension or to thrombin and stimulation with various growth factors and cytokines (cf. Heldin and Westermark, 1999). Furthermore, PDGF levels have been shown to be elevated in several CNS disorders associated with fever such as trauma, stroke, meningitis, cerebral abcesses, glial and meningeal cysts and neoplasia (Iihara et al., 1994; Nister et al., 1994).

Multiple mechanisms are responsible for fever, and considerable evidence suggests that the generation of fever involves the synthesis by immunocompetent cells of inflammatory mediators of protein origin, such as cytokines, and

those of lipid origin that are derived from the metabolism of cell membrane lipids, such as prostaglandins (Kluger, 1991). Recently, Pela et al. (1998, 2000) showed that microinjection of PDGF-BB into the lateral ventricle induced a febrile response in rats that was reduced by pretreatment with Win 41662, a potent inhibitor of PDGF receptors. Win 41662 also blocked lipopolysaccharide-induced fever, suggesting that endogenous PDGF also participates in the febrile response to this exogenous pyrogen.

The many physiological events associated with the production of NO include neuronal transmission, the regulation of blood pressure, the regulation of cellular proliferation and immune-mediated cellular toxicity (for review, see Moncada et al., 1991). NO has also been shown to affect thermoregulation and fever (Steiner and Branco, 2001b). The enzymes responsible for endogenous NO synthesis, NOS, are widely distributed throughout the body (Dawson and Snyder, 1994; Moncada et al., 1991). Because NOS is encountered in various tissues involved in the regulation of body temperature (cf. Steiner and Branco, 2001b) and of the immune system (Singh et al., 2000), it seems probable that this gas influences thermoregulation and fever. In this context, it has been demonstrated that systemic administration of NOS inhibitors impairs the fever produced by endotoxin in rats and guinea pigs (Kamerman and Fuller, 2000; Roth et al., 1998a) by muramyl dipeptide in guinea pigs (Kamerman and Fuller, 2000), and by yeast (Ataoglu et al., 2000) and interleukin-1 (Reimers et al., 1994; Roth et al., 1998b) in rats. Furthermore, recent studies reported that systemically administered L-NAME also impairs restraint- and open field-induced fever (De Paula et al., 2000). On the other hand, i.c.v. treatment with NOS inhibitors has been shown to enhance endotoxin fever in rats (Almeida et al., 1999) and rabbits (Gourine, 1995). Moreover, endotoxin-tolerant animals develop a greater febrile response when treated with NO synthesis inhibitors (Almeida et al., 1999; Furfine et al., 1995). L-NAME was also shown to enhance the rise in body temperature evoked by restraint (De Paula et al., 2000), supporting the hypothesis that NO acts as an endogenous antipyretic in the CNS. These data indicate that the fever-inhibiting effect of systemically administered NOS inhibitors is due to a peripheral action of these drugs. Taken together, these data support the notion that NO has differential thermoregulatory effects whether acting at the periphery or in the central nervous system, with peripheral NO being a pyretic molecule and central NO an antipyretic molecule (Steiner and Branco, 2001b). In the present study, we used i.c.v. injections of the nonselective NOS inhibitor, L-NAME. Our data show that i.c.v. treatment with L-NAME exacerbated PDGF-BB-induced fever, corroborating the notion that NO has an antipyretic role in the CNS, not only in lipopolysaccharide-induced fever, but also in fevers elicited by other stimuli such as restraint (De Paula et al., 2000; Sanches et al., 2002) and PDGF-BB (present study).

Aminoguanidine, a molecule containing the guanidino group of L-arginine linked to hydrazine, was identified in 1992 as one of the first selective inhibitors of iNOS (Corbett et al., 1992). Aminoguanidine is at least equipotent to N_{ω}-monomethyl-L-arginine (L-NMMA—a nonselective NOS inhibitor) to inhibit iNOS, but lacks activity against nNOS and eNOS (Corbett et al., 1992). Thus, we used aminoguanidine to verify if iNOS is involved in PDGF-BB fever in rats. It was observed that i.c.v. aminoguanidine did not affect the body temperature of euthermic animals. Moreover, i.c.v. aminoguanidine caused no change in the course of PDGF-BB-induced fever (Fig. 3), suggesting that iNOS is not the NOS isoform involved in PDGF-BB fever. Considering that the overexpression of iNOS mRNA requires 3–6 h to be induced (Mustafa and Olson, 1998) and that PDGF-BB fever was evident 1 h after the injection, this result is not surprising. In agreement with this, aminoguanidine has been reported to have no effect on endotoxin fever in guinea pigs (Roth et al., 1999) and to have little effect on IL-1-induced fever in rats (Reimers et al., 1994). In contrast, aminoguanidine seems to impair muramyl dipeptide-induced fever (Kamerman and Fuller, 2000), even though this fever also takes about 1 h to start.

Because our data indicate that iNOS is not the NOS isoform involved in PDGF-BB fever in rats, we hypothesized that nNOS is involved in PDGF-BB-induced fever. We then used SMTC, which has been shown to be a more selective nNOS inhibitor (Furfine et al., 1994). Fig. 4 shows that under the present experimental conditions, i.c.v. injection of SMTC caused no change in the body temperature of euthermic rats, but significantly enhanced PDGF-BB-induced fever, indicating that the nNOS isoform plays no role during euthermia but does play an important role in the development of PDGF-BB-induced fever. Interestingly, it has been shown that nNOS is the NOS isoform involved in restraint stress-induced fever (Sanches et al., 2002). It should be emphasized that the effect of i.c.v. SMTC on PDGF-BB-induced fever was less than from that observed for i.c.v. L-NAME (Fig. 2). This could be due to a difference in the potency of these drugs to inhibit nNOS. However, a possible participation of eNOS cannot be excluded. To our knowledge, no eNOS selective inhibitor is commercially available to date. The use of NOS knockout animals could be a useful tool to solve this problem.

Recent studies have demonstrated that NO is involved in some actions elicited by PDGF. In this context, it has been demonstrated that vascular smooth muscle cell proliferation is attenuated by NO (Sandirasegarane et al., 2000). Moreover, hypoxia-induced PDGF expression is inhibited by nitric oxide (Kourembanas et al., 1993). Furthermore, it has been demonstrated that PDGF-induced prostaglandin E_2 release and increase in prostaglandin H_2/E_2 isomerase activity is inhibited by NO (Kelner and Uglik, 1994). The present study adds NO as a mediator of the febrile response induced by PDGF-BB in rats.

Recently, the gaseous compound, CO, has been shown to act as a neurotransmitter or neuromodulator (Dawson

and Snyder, 1994; Johnson et al., 1997). Studies have suggested that CO arising from heme, via HO metabolism, stimulates soluble guanylate cyclase activity and promotes an increase in cGMP in neural tissue (Johnson et al., 1997). The physiological importance of the HO pathway can be demonstrated by the inhibition of the enzyme, HO, using metalloporphyrins such as ZnDPBG (Johnson et al., 1997).

HO has been found to be expressed in the CNS of several species, including rats (Ewing et al., 1992; Kitamura et al., 1998) and humans (Takahashi et al., 1996). Accordingly, evidence has accumulated that the HO pathway has a substantial role in the control of blood pressure (for review, see Johnson et al., 1997) and of neuroendocrine function (Mancuso et al., 1998). In spite of the growing evidence showing the participation of CO in several physiological responses, it was only recently that a thermoregulatory effect of CO was assessed. Inhibition of the HO/CO pathway in the CNS has been shown to exacerbate insulin-induced hypothermia in rats (Almeida and Branco, 2002), indicating that the HO/CO pathway acts by preventing excessive drops in body temperature. Furthermore, i.c.v. injection of ZnDPBG has been shown to attenuate the rise in body temperature evoked by endotoxin (Steiner and Branco, 2001a). Heme overload, which induces the HO pathway (Takahashi et al., 1996), produces a rapid rise in body temperature when applied to the brain, a response that is attenuated by pretreatment with ZnDPBG (Steiner and Branco, 2001a). In the present study, rats injected with ZnDPBG showed no changes in body temperature, indicating that the central HO pathway does not play a tonic role in the maintenance of euthermic animals under the experimental conditions used in the present study. Moreover, in our experiments, i.c.v. injection of ZnDPBG did not affect the rise in body temperature induced by PDGF-BB, suggesting that the HO/CO pathway in the CNS plays no role in PDGF-BB-induced fever.

In a previous study from our laboratory (Steiner and Branco, 2000), it has been shown that the rise in body temperature evoked by overload of heme is not altered by treatment with indomethacin, which inhibits cyclooxygenase, indicating that the CO/HO pathway in the CNS elicits hyperthermia in a prostaglandin-independent way. Conversely, PDGF-BB-induced fever has been shown to be blocked by indomethacin (Pela et al., 2000), suggesting that a cyclooxygenase-dependent pathway may be involved in this process. These two studies are in agreement with the present data that show that CO has no effect on PDGF-induced fever.

In summary, the present data indicate that only the NO pathway, but not the HO/CO pathway, modulates PDGF-BB-induced fever in the rat CNS. Furthermore, our results show that nNOS is the NOS isoform responsible for NO synthesis in this response. It is important to emphasize that this mechanism is different from that proposed for lipopolysaccharide fever where both NO and CO pathways have been shown to participate. Understanding of the central mechanisms involved in fever induced by different stimuli is essential not only for the understanding of animals' strategies to regulate body temperature under different conditions, but also, it gives evidence related to the systemic reactions developed by the host to protect itself from deleterious effects of the invader agent, during experimental and clinical conditions when natural barriers are broken.

Acknowledgements

We thank C. Fernando Valenzuela (University of New Mexico, HSC) for critically reading the manuscript. We also thank Mauro F. Silva and Daniela L. Oliveira for their excellent technical assistance. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and PRONEX. M.C. Almeida is the recipient of a CAPES graduate scholarship.

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