Carbon monoxide as a novel mediator of the febrile response in the central nervous system

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Steiner, Alexandre A., Eduardo Colombari and Luiz G. S. Branco. Carbon monoxide as a novel mediator of the febrile response in the central nervous system. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R499–R507, 1999.—Heme oxygenase catalyzes the metabolism of heme to biliverdin, free iron, and carbon monoxide (CO), which has been shown to be an important neuromodulatory agent. Recently, it has been demonstrated that lipopolysaccharide (LPS) can induce the enzyme heme oxygenase in glial cells. Therefore, the present study was designed to test the hypothesis that central CO plays a role in LPS-induced fever. Colonic body temperature (Tc) was monitored in awake, unrestrained rats (basal Tc = 36.8 ± 0.2°C). Intracerebroventricular injection of zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG; 75 nmol), a heme oxygenase inhibitor, caused a significant increase in Tc, indicating that the central heme oxygenase pathway plays a major role in Tc under the experimental conditions used. Intraperitoneal injections of LPS (50–100 µg/kg) caused a dose-dependent increase in Tc. Intracerebroventricular injection of ZnDPBG in febrile rats attenuated LPS-induced fever (thermal index with ZnDPBG = 1.1 ± 0.2°C, thermal index with vehicle = 2.3 ± 0.4°C), suggesting that the central heme oxygenase pathway plays a role in fever generation. The antipyretic effect of ZnDPBG could be reversed by intracerebroventricular administration of heme-lysinate or CO-saturated saline. Collectively, our data indicate that CO arising from heme oxygenase may play an important role in fever generation by acting on the central nervous system.

FEVER IS A PHENOMENON characterized by a raised thermoregulatory set point, which leads to an elevation in body temperature (20). Fever can be a beneficial response to infections because it enhances the immune response (20). The importance of this response is emphasized by reports showing that moderate fever has a beneficial effect on the outcome of infections. Infected lizards, goldfish, and newborn mammals have higher survival rates when febrile (see Ref. 20). Clinical studies have also shown this correlation (see Ref. 20). Moreover, suppression of fever using antipyretic drugs results in an increased mortality rate in bacterially infected rabbits (42).

It is well known that fever can be initiated by a number of agents, including endotoxin (LPS), viruses, and gram-positive bacteria (20). Considerable efforts have been made to identify the mechanisms of fever, and evidence suggests that a common pathway underlies fever during a variety of disorders. This common mechanism is believed to involve induction of cytokines, such as interleukin (IL)-1β, IL-6, interferons, and tumor necrosis factor (20), and subsequent stimulation of the generation of prostaglandins in the central nervous system, particularly PGE2, which is known to act as one of the final mediators of fever (30). In addition to this conventional viewpoint, recent evidence has accumulated that distinct mechanisms may be evoked, depending on the route of pyrogen administration (10) and the sort of pyrogen injected (45).

Recently, a new neuromodulatory agent, the gaseous compound carbon monoxide (CO), has been shown to play a role as neurotransmitter or neuromodulator (7). Heme oxygenase, the enzyme responsible for CO synthesis in vivo, catalyzes the metabolism of heme to biliverdin, free iron, and CO (1, 24, 31). Three distinct heme oxygenase isoforms have been identified (see Ref. 25): heme oxygenase-1, heme oxygenase-2, and heme oxygenase-3, among which the isoforms 1 and 2 are the most studied and known (8). Both isoenzymes, one of them inducible (heme oxygenase-1) and the other constitutive (heme oxygenase-2), are expressed in various tissues, including neural tissue (23, 27). Studies have suggested that CO arising from heme via metabolism by heme oxygenase stimulates soluble guanylate cyclase activity and promotes an increase in cGMP in neural and cardiovascular functions (24, 32). Additionally, subsequent studies have revealed that CO may also exert biological activities via alternative pathways, such as the activation of cyclooxygenase (26), which is known to participate in fever generation by acting on the central nervous system (30).

Interestingly, an in vitro study demonstrated that the heme oxygenase-1 isoform is induced in rat glial cells culture treated with LPS (17). Moreover, it has been demonstrated in vivo that LPS injected into the hippocampus also induces heme oxygenase-1 (16). Cytokines have also been shown to induce heme oxygenase in the liver and are likely to mediate the effect of LPS on the enzyme (35). However, to our knowledge, no reports are available about the effect of CO on LPS-induced fever.

In view of these considerations, the aim of the present study was to assess the role of the central heme-heme oxygenase-CO pathway in LPS-induced fever in rats by using the nonselective heme oxygenase inhibitor zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG), the heme oxygenase substrate heme-lysinate, and its product CO.
METHODS

Animals. Experiments were performed on adult male Wistar rats weighing 250–300 g, housed at controlled temperature (25.3 ± 1.1°C) and exposed to a daily 12:12-h light-dark cycle with lights on at 6:00 AM. The animals were allowed free access to water and food. Experiments were performed between 9:00 AM and 4:00 PM.

Drugs. The nonselective heme oxygenase inhibitor ZnDPBG was obtained from Porphyrin Products, and CO was from White Martins (Brazil). ZnDPBG (18.75 µmol/ml) was dissolved in 50 mmol/l Na₂CO₃ and stored in the dark. CO-saturated saline was prepared according to the procedure described by Johnson et al. (14). Five milliliters of sterile saline was placed in a 10-ml vial fitted with a small aperture and bubbled at 4°C with CO gas for 20 min. The vial was then tightly sealed and warmed at room temperature before use. LPS from Escherichia coli (serotype O111:B4) was obtained from Sigma Chemical and dissolved in pyrogen-free sterile saline. Heme-lysinate (38 mmol/l) was prepared as previously described (22, 41). Heme-free preparations were used as amino acid (L-lysine) vehicle control solutions.

Surgery. Animals were anesthetized with 2,2,2-tribromoethanol (Aldrich, Milwaukee, WI) and fixed in a stereotaxic frame. A stainless steel guide cannula (0.7 mm OD) was introduced into the right lateral cerebral ventricle (coordinates: anterior –1.0 mm, lateral –1.6 mm, dorsal 3.2 to 3.7 mm) (33). 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 surgical procedures were performed over a period of 30 min. After surgery, animals were treated with 100,000 U of benzyl-penicillin and allowed to recover for 1 wk after cannula placement.

Determination of the temporal effect of intraperitoneal LPS injection on body temperature. Rats were housed in a plastic chamber (5 liters) for at least 24 h before control body temperature (Tb) was measured by inserting a thermoprobe (Cole Parmer, model 8502–10, Chicago, IL) 4 cm into the colon each time Tb was to be measured. The animals were held manually and gently during Tb determination, a procedure that lasted no more than 1 min. Control Tb was determined by four measurements at 30-min intervals. It should be pointed out that, for all protocols, 2 days before the experiment the animals were habituated to temperature measurements that were performed quickly to avoid any stress-induced elevations in Tb. The animals were then treated with LPS (E. coli, Sigma) dissolved in pyrogen-free sterile saline by intraperitoneal injection of 50 or 100 µg/kg body wt and Tb was measured every 30 min for 4.5 h. The volume of each injection was 0.5 ml. Control animals received intraperitoneal injections of saline (0.5 ml). In all experimental protocols each rat was used only once.

Fever indexes were used to improve data analysis. They were calculated as areas under the fever curves (°C/h) for a total period of 4.5 h.

Determination of the central effect of the heme oxygenase inhibitor ZnDPBG on Tb. Rats previously cannulated in the lateral ventricle were housed in a plastic chamber (5 liters) for at least 24 h before the experiment. After control Tb was measured rats were treated with ZnDPBG by intracerebroventricular injection of 75 nmol, and Tb was measured every 30 min for 2 h. Na₂CO₃, 50 mmol/l (vehicle), was injected as control. The volume of each injection was 4 µl for all protocols. This dose was used because, when preliminary doses of ZnDPBG were tested, the thermoregulatory response to the dose of 75 nmol icv was the most consistent and repeatable in all our experimental protocols.

A 10-µl Hamilton syringe and a dental injection needle (Missy, 200 µm OD) were used for the intracerebroventricular injections. Injection was performed over a period of 2 min, and 1 min was allowed to elapse before the injection needle was removed from the guide cannula to avoid reflux.

Determination of the central effect of the heme-lysinate preparation on Tb. Rats previously cannulated in the lateral ventricle were left undisturbed for at least 24 h before the experiment, after which control Tb was measured. Rats were then treated intracerebroventricularly with heme-lysinate (152 nmol in 4 µl) or the same volume of the L-lysine-vehicle mixture. Tb was measured every 30 min for 2 h after the injections. This dose was used because, when preliminary doses of the drug were tested, the thermoregulatory response to the dose of 152 nmol icv was the most consistent and repeatable.

In another set of experiments, basal Tb was measured and animals were injected intracerebroventricularly with ZnDPBG (75 nmol/4 µl) or its vehicle. Five minutes later, heme-lysinate (152 nmol/4 µl) was injected into the lateral ventricle of both groups and Tb was measured every 30 min for a total period of 2 h. Fever indexes were calculated as areas under the curves (°C/h) after the treatments for a total period of 2 h.

Determination of the central effect of exogenous CO on Tb. Rats previously cannulated were left undisturbed for at least 24 h. After control Tb was determined, the animals were treated intracerebroventricularly with 4 µl of CO-saturated saline and Tb was measured every 15 min for a 1-h period. Control animals received intracerebroventricular injections of the same volume of pyrogen-free sterile saline only.

Determination of the role of the central heme-heme oxygenase pathway in LPS-induced fever. Control Tb was determined, and rats were then injected intraperitoneally with LPS (100 µg/kg body wt). Tb was then measured every 30 min for a total period of 2.5 h, when the first significant rise in Tb was observed. After this period, the nonspecific heme oxygenase inhibitor ZnDPBG (75 nmol/4 µl) or its vehicle (Na₂CO₃, 50 mmol/l) was injected intracerebroventricularly and Tb was measured every 30 min for 2 h. Because in pilot experiments we have observed that ZnDPBG at the dose used in the present study affects fever for ~2 h, we chose to inject ZnDPBG just after the rise in Tb (2.5 h after LPS injection).

In additional experiments, all animals were preinjected intracerebroventricularly with heme-lysinate (152 nmol/4 µl) or the same volume of vehicle 2.5 h after LPS injection. Five minutes after heme-lysinate or its vehicle was injected intracerebroventricularly, ZnDPBG (75 nmol/4 µl) was injected in both groups and Tb was measured every 30 min for a total period of 2 h. To analyze the effect of central ZnDPBG and heme-lysinate/ZnDPBG on LPS-induced fever, we calculated thermal indexes (TI) for the fever curves obtained after intracerebroventricular injections (between 2.5 and 4.5 h after LPS injection). These 2-h TI (°C/h) were calculated as areas under the fever curves.

Determination of the role of central CO in LPS-induced fever. In further experiments, LPS (100 µg/kg body wt) was injected intraperitoneally, fever was allowed to develop for 2.5 h, and ZnDPBG (75 nmol/4 µl) was injected intracerebroventricularly. One hour after ZnDPBG injection, when the maximum effect of the drug was observed, rats were injected with CO-saturated saline, and Tb was measured every 15 min.
for a total period of 1 h. Pyrogen-free sterile saline (4 µl) was injected as control.

Statistical analysis. All values in this study are reported as means ± SE. Changes in T_b were evaluated by ANOVA for repeated measurements. The difference between means was assessed by the Tukey-Kramer multiple comparisons test. Ordinary ANOVA or Student’s t-test was used to assess differences between TIs. Values of P < 0.05 were considered to be significant.

RESULTS

In all experimental protocols, T_b ranged from 36.5 to 37.1°C (mean ± SE = 36.8 ± 0.2°C) during the control period, and the baseline values of the experimental groups did not differ significantly from those of the control group. During the experiments, room temperature was 26.2 ± 0.6°C.

Temporal effect of the intraperitoneal injection of LPS on T_b. Figure 1 shows the effect of intraperitoneal LPS injection on T_b. LPS evoked a fever that started to increase 2 h after the injections, whereas saline caused no significant change in T_b. A slight but not significant increase in T_b was observed 30 min after all injections and is probably due to handling and injections. TIs showed that LPS at the dose of 100 µg/kg (TI = 2.7 ± 0.8°C/h), but not at the dose of 50 µg/kg (TI = 1.7 ± 0.9°C/h), caused a significant increase in T_b compared with the group injected with saline (TI = 0.4 ± 0.1°C/h). Thus the dose of 100 µg/kg was chosen for further experiments.

Temporal effect of intracerebroventricular injection of ZnDPBG on T_b. No significant change in T_b was observed after intracerebroventricular injection of ZnDPBG or its vehicle. These data are shown in Fig. 2.

Temporal effect of intracerebroventricular injection of hemolyamine on T_b. Figure 3 shows the effect of heme-lysinate injected into the cerebral ventricle on T_b. Heme-lysinate evoked an elevation in T_b (TI = 1.8 ± 0.4°C/h), whereas the L-lysine-vehicle mixture caused no significant change in T_b (TI = 0.5 ± 0.1°C/h). ZnDPBG administration significantly attenuated the increase in T_b elicited by the heme preparation with a TI = 0.8 ± 0.1°C/h, which is not significantly different from the group treated with heme-lysinate vehicle only (control). Moreover, intracerebroventricular treatment with the ZnDPBG vehicle (Na2CO3) did not alter the rise in T_b induced by heme-lysinate (TI = 2.0 ± 0.4°C/h).

Effect of intracerebroventricular injection of ZnDPBG on LPS-induced fever. Figure 4 shows the effect of intracerebroventricular injection of the heme oxygenase inhibitor ZnDPBG (75 nmol) on fever induced by intraperitoneal injection of LPS (100 µg/kg body wt). No difference was observed between fever curves before ZnDPBG injection, but ZnDPBG significantly (TI = 1.1 ± 0.2°C/h, P < 0.05) attenuated LPS-induced fever compared with the group treated with ZnDPBG vehicle (TI = 2.3 ± 0.4°C/h).

Additionally, Fig. 5 shows that the central antipyretic effect of ZnDPBG was reversed by treatment with the heme preparation (TI = 2.5 ± 0.2°C/h). Intracerebroventricular injection of L-lysine-saline did not modify the effect of ZnDPBG (TI = 1.7 ± 0.3°C/h).
Effect of intracerebroventricular injection of CO-saturated saline on $T_b$ of euthermic and febrile rats treated with ZnDPBG. Figure 6A shows the effect of centrally administered CO-saturated saline or only saline as control on $T_b$ of euthermic rats. A significant ($P < 0.05$) increase in $T_b$ was observed at 45 min and 1 h after CO-saturated saline injection, whereas treatment with saline caused no significant change in $T_b$.

Similar to the results shown in Fig. 6A, intracerebroventricular injections of CO-saturated saline caused an increase in $T_b$ of animals pretreated with LPS (100 µg/kg body wt ip) followed by ZnDPBG (75 nmol icv). Saline alone did not alter the effect of ZnDPBG. These data are plotted in Fig. 6B.

DISCUSSION

The present study provides evidence that CO arising from heme metabolism by the enzyme heme oxygenase in the central nervous system may play an important role in fever generation because intracerebroventricular administration of ZnDPBG, a competitive inhibitor of the enzyme heme oxygenase, reduced LPS-induced fever. This effect could be reversed by intracerebroventricular administration of the heme oxygenase substrate heme-lysinate or CO-saturated saline. Moreover, the present study also provides evidence that the central heme oxygenase pathway plays no tonic role in body temperature regulation.

Fever is a common manifestation of injury, tumor, infection, and inflammation. Most evidence supports the hypothesis that the febrile increase in body temperature in response to infections is beneficial, serving to protect the host by improving the efficiency of macrophages in killing invading agents and impairing the replication of many microorganisms (see Ref. 36). Multiple mechanisms are responsible for fever, and considerable evidence suggests that generation of fever involves de novo synthesis 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 (see Refs. 20 and 30).

New efforts have been made to elucidate the mechanisms by which cytokines released in response to
Peripheral infection signal the brain to elicit fever. More than 10 years ago, the first papers were published suggesting that the organum vasculosum laminae terminalis (OVLT), one of the circumventricular organs with an incomplete blood-brain barrier, is the site of entry of circulating cytokines into the brain (4, 39). Another possible mechanism is that large hydrophilic cytokine proteins might enter the brain by specific saturable transport systems (2). Recently, it has been demonstrated that vagotomized animals develop lower fever (see Ref. 10), suggesting that vagal afferent fibers play a crucial role in the transduction of immune signals to the brain. Moreover, it has been shown that the pathway by which the signal is transduced to the brain depends on the route of administration (10). This last hypothesis is supported by the finding that fever elicited by intraperitoneal injection of LPS, but not by intramuscular injection, is suppressed by subdiaphragmatic vagotomy in guinea pigs. Thus it should be emphasized that in the present study we examined the central role of CO in fever induced by intraperitoneal injection of LPS (Fig. 1).

Peripherally administered LPS is not only capable of stimulating the release of cytokines from activated immune cells in peripheral tissues but can also increase the levels of cytokines and prostaglandins in various brain areas. Accordingly, it has been suggested that IL-1β could act in the brain, namely in the anterior hypothalamus, to produce fever (21). Indeed, bioactive IL-6, another pyrogenic cytokine, is measurable in the hypothalamus in response to peripheral injection of LPS (19). PGE2, a nonprotein mediator of fever, has been suggested to act directly in the preoptic area of the anterior hypothalamus as well as in the OVLT (28), causing an increased thermoregulatory set point, i.e., fever. Although the central mechanisms of fever have been extensively studied, they remain insufficiently understood.

A number of recent studies have implicated the gaseous compound CO as a neurotransmitter or neuromodulator (7). Heme oxygenase is the enzyme responsible for CO synthesis in vivo and seems to be exten-

Fig. 5. A: change in Tb over time of rats injected intraperitoneally at time zero with LPS at the dose of 100 µg/kg. Two and a half hours after LPS injection, rats were pretreated intracerebroventricularly with heme preparation (152 nmol) or its vehicle followed by an intracerebroventricular injection of ZnDPBG (75 nmol). Arrows, times of injection. Values are means ± SE, n = 6 per group. *Significant (P < 0.05) difference between 2 groups.

Fig. 6. A: change in Tb over time of rats injected intracerebroventricularly at time zero with carbon monoxide (CO)-saturated saline or saline alone. Arrow, time of injection. CO-saturated saline lead to a significant increase in Tb. Values are means ± SE, n = 7 per group. *Significant (P < 0.05) difference compared with control value. B: change in Tb over time of rats injected intracerebroventricularly with CO-saturated saline or saline and pretreated with LPS (100 µg/kg ip) and ZnDPBG (75 nmol icv). Arrow, time of injection. CO-saturated saline led to a significant increase in Tb. Values are means ± SE, n = 6 per group. *Significant (P < 0.05) difference compared with control value.

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sively distributed throughout the body, including the central nervous system (23, 27). This enzyme catalyzes the oxidation of the heme molecule in concert with NADPH-cytochrome P-450 reductase, with resulting specific cleavage of the heme molecule into biliverdin, free iron, and CO (17). The physiological importance of the heme-heme oxygenase pathway can be demonstrated by the inhibition of the enzyme heme oxygenase using metalloporphyrins such as ZnDPBG (14, 44). In the present study, we have chosen ZnDPBG because it is a potent and nonselective inhibitor of heme oxygenase, acting on both the constitutive and inducible isoforms of the enzyme (14, 15, 44).

To our knowledge, no report is available about the role of the heme oxygenase pathway in thermoregulation. In the present study, rats treated intracerebroventricularly with ZnDPBG showed no change in $T_b$ (Fig. 2), indicating that the central heme oxygenase pathway does not play a tonic role in the maintenance of $T_b$ in euthermic animals under the experimental conditions used in the present study. However, the heme oxygenase pathway could play a role in other situations, such as the nighttime rise in rat $T_b$.

We also demonstrated that central administration of heme-lysinate elicits a rise in $T_b$ (Fig. 3). A previous report (15) showed that the substrate heme-lysinate causes an increase in the activity of the enzyme heme oxygenase. Accordingly, the rise in $T_b$ after heme-lysinate intracerebroventricular injection could be due to an increase in the production of a heme oxygenate product or just to a direct effect of the heme group. If the increase in $T_b$ observed after heme administration is consequent to the formation of heme oxygenate products, inhibitors of heme oxygenase activity would be expected to interfere with this effect. Indeed, centrally administered ZnDPBG attenuated the heme-lysinate-induced rise in $T_b$ (Fig. 3), indicating that a heme oxygenate product causes an increase in $T_b$ by acting on the central nervous system in rats. Moreover, the heme oxygenate product CO injected intracerebroventricularly led to a significant increase in $T_b$ (Fig. 6A). Taken together, these findings suggest that a heme oxygenate product, probably CO, has the effect of increasing $T_b$ in rats by acting on the central nervous system, but more studies are necessary to assess whether this effect is a regulated rise in $T_b$ (fever) or just hyperthermia. It is important to point out that the increase in $T_b$ elicited by CO injection was less pronounced than that induced by the heme preparation, a fact possibly due to 1) a lower amount of CO delivered to the brain, 2) a rapid diffusion of the gas out of the brain because this gas has a high affinity for hemoglobin, or 3) the fact that, besides CO, another heme oxygenate product is involved. Furthermore, we also observed that the increase in $T_b$, after administration of CO-saturated saline occurred later than after the heme preparation injection. We are unable to clearly explain this difference, but on the basis of the reasons listed above we speculate that it could be due to a lower amount of CO delivered to the brain by the CO injection, which would be less effective in inducing the mechanisms that culminate in the elevated $T_b$. More studies are needed to clarify this issue.

Interestingly, in our experiments, intracerebroventricular injection of ZnDPBG reduced the magnitude of LPS-induced fever (Fig. 4), an event reversed by treatment with the heme (substrate) preparation (Fig. 5), suggesting that a heme oxygenase product in the central nervous system plays an important role in fever generation. The heme oxygenase pathway may play a role in fever generation by acting on several brain areas that are activated after peripheral administration of LPS, among them the hypothalamus (13, 20), OVLT (4, 11), brain stem (10, 14), thalamus, and hippocampus (3, 34). A recent study has indicated that at least the inducible heme oxygenase isoform is unlikely to act in the hypothalamus to produce fever because it has been observed that the enzyme heme oxygenase-1 messenger RNA is not induced in rat hypothalamus after peripheral administration of endotoxin (13). However, this does not exclude a possible role of the heme oxygenase-2 (constitutive). Besides the hypothalamus, the OVLT has been shown to play a major role in fever generation (4), with cytokines possibly acting through the production of nitric oxide and prostaglandins (11). No report exists about the role of CO arising from heme oxygenase in the OVLT. Moreover, it has been demonstrated that heme oxygenase has important physiological functions in the brain stem (14), an area that has been shown to be essential to the induction of fever via subdiaphragmatic vagal afferents (10). Other brain areas, such as thalamus and hippocampus, that express the enzyme heme oxygenase and are activated after peripheral injection of LPS, could also be involved (3, 34). Further studies are needed to firmly establish the specific sites in the central nervous system where the heme oxygenase pathway might be exerting its effects.

Cytokines have been shown to induce heme oxygenase in vitro and in vivo, a fact that has been inferred to mediate LPS-induced heme oxygenase activation (35). Experiments with LPS-resistant C3H/HeJ mice confirmed that induction of heme oxygenase mRNA after LPS was due to mediator molecules rather than to LPS itself, because LPS was not so effective as in normal mice. Because the mechanism of LPS resistance of the C3H/HeJ mouse strain is thought to be a defective response of macrophages to LPS, it is likely that cytokines produced by macrophages are the mediators involved. Actually, IL-1 has been demonstrated to be extremely potent in inducing heme oxygenase mRNA (35). However, to our knowledge, this evidence has been obtained in studies on peripheral tissues, mainly the liver, and no report is available about the induction of heme oxygenase by cytokines in the central nervous system.

All the products of heme oxygenase have been implicated in physiological actions. Iron can stimulate the generation of free radicals and promote lipid peroxidation (43) or induce guanylate cyclase by a direct action (37). Biliverdin and its derivative bilirubin are antioxidative and as such may act by affecting oxidative...
processes (40), including those dependent on nitric oxide. Finally, CO can act as a gaseous neurotransmitter or neuromodulator, similar to nitric oxide (7), known to participate in thermoregulation (5, 11, 38).

In fact, our data indicate that the antipyretic effect of ZnDPBG may be a consequence of decreased production of CO into the brain, because injections of CO into a lateral ventricle reversed, at least partially, the effect of ZnDPBG (Fig. 6B). This reversal was partial, probably because of the same reasons listed for the effects of centrally applied CO in euthermic conditions. Interestingly, the CO-induced elevation in Tb seems to be more consistent during fever (Fig. 6B) compared with the effect during euthermic conditions (Fig. 6A). Despite this evidence, CO-saturated saline significantly increased Tb 60 min after its injection in febrile rats pretreated with ZnDPBG, whereas CO caused a significant elevation in Tb 45 min after its injection in euthermic rats. We believe that this small timing difference is irrelevant and might be the result of variation among the experimental groups.

The exact way CO acts in the brain is unknown, but several considerations may be mentioned. The mode of action of CO is exquisitely paracrine, because the gas acts only at a short distance from its sites of generation (7). It has been shown that CO, in most cases, produces an elevation of cGMP levels (24, 32). In addition, another signaling pathway, the cyclooxygenase pathway, has been observed in some cases (26). CO can also inhibit electron transport into mitochondria and thus interfere with ATP production (6). Moreover, CO has been reported to be a potential messenger molecule for the excitatory amino acid glutamate (7). This adds to the interaction between CO and nitric oxide, which has also been suggested to act on glutamate-mediated events (7). In fact, some studies (18, 29) have already provided evidence that CO can bind to nitric oxide synthase and inhibit the production of nitric oxide. In addition, an in vitro study (17) demonstrated that nitric oxide induces the heme oxygenase-1 protein in microglia and astrocytes exposed to LPS, suggesting a coordinated interaction between nitric oxide and CO production. However, the exact mode of action of central CO during fever has still to be assessed.

In summary, our data indicate that CO arising from the metabolism of heme by the enzyme heme oxygenase in the central nervous system may play an important role in fever generation (Fig. 7). However, it is not clear from the present study the heme oxygenase subtype involved because we used a nonselective heme oxygenase inhibitor. Moreover, the cell types responsible for CO production during fever, i.e., glial cells, neurons, or endothelial cells, remain to be determined. More studies are certainly needed to clarify these issues.

**Perspectives**

The discovery of the endothelium-derived relaxing factor by Furchgott and Zawadzki in 1980 (9), further identified as the gas nitric oxide (12), certainly started a revolution in the understanding not only of blood pressure control but also of other physiological or pathophysiological conditions, including thermoregulation (5, 11, 38). We now report that the gaseous compound CO may play a role in fever generation by acting on the central nervous system, which may create a new area of research investigation into the mechanisms of fever. Actually, CO has been shown to alter neuronal activity, neuroendocrine function, and nitric oxide synthesis, which have been implied to be involved in the febrile response, but the exact actions of CO during fever remain to be determined.

Fever is a multimediated process that is characterized by a raised thermoregulatory set point, which leads to an elevation in Tb (20). The febrile increase in Tb by a few degrees has been inferred to be protective because it enhances the immune response. However, it is critical to the host that Tb does not get too close to lethal limits. Clearly, pharmacological modulation of the action or production of the complex systems of mediators involved in fever generation is of clinical significance. We have shown that the heme oxygenase inhibitor ZnDPBG, acting on the central nervous system, attenuates fever. Because ZnDPBG is known to cross the blood-brain barrier (cf. 15, 44), this drug could lead to the development of a new class of antipyretic drugs with clinical applications. Actually, hemoglobinase inhibitors such as tin protoporphyrin and zinc protoporphyrin have already been used in the treatment of physiological jaundice in human neonates (44). However, there are still some difficulties regarding the clinical use of the heme oxygenase inhibitors. First, most of these inhibitors are photosensitizers (44). Second, the systemic inhibition of heme oxygenase, at least in rats, leads to an increase in blood pressure (15). The development of more selective inhibitors of the enzyme heme oxygenase, with fewer side effects, may be defi-
nity useful for the development of new antipyretic drugs with clinical application.

We thank Mauro F. Silva for the excellent technical assistance.

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico. A. A. Steiner was the recipient of a FAPESP fellowship.

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Received 3 March 1999; accepted in final form 29 April 1999.

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