Role of nitric oxide in the cerebrovascular and thermoregulatory response to interleukin-1β

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In the CNS, the cells that have been found to be capable of NO production include astrocytes, microglia, neurons, and endothelial cells. Neurons containing NOS have been identified in brain regions that participate in thermoregulation, such as the hypothalamic nuclei and circumventricular organs, suggesting that NO may play a central role in the regulation of temperature (3, 30, 39, 46). Inhibition of NOS in experimental animal models of sepsis has attenuated the height and duration of fever, providing indirect evidence that NO mediates the febrile response to IL-1β (15, 34, 37). However, the exact role of NO in thermoregulation has not been clearly delineated (40).

Because the effect of temperature on the cerebrovascular response to IL-1β has not been previously investigated, we designed the present study to investigate the relationship between the cerebrovascular and thermoregulatory responses to central injection of IL-1β, and to determine the role of NO in these responses. We hypothesized that 1) central injection of IL-1β increases cortical CBF (cCBF) and brain and body temperature, 2) the vasodilatory effects of IL-1β are independent of temperature, and 3) inhibition of NOS by

INTERLEUKIN-1β (IL-1β), a 17-kDa polypeptide possessing a wide spectrum of inflammatory, metabolic, physiological, hematopoietic, and immunologic activities, is produced by a variety of cells, including lymphocytes, mononuclear phagocytes, and endothelial cells (13). Intracerebroventricular (icv) injection of IL-1β causes pituitary adenoid activation, hyperinsulinemia, slow-wave sleep, behavioral changes, hypophagia, glial proliferation, neovascularization, peripheral neutrophilia, and fever (6, 10–13, 22, 31, 35). In a normal brain, low amounts of IL-1β have been identified in microglia, astrocytes, and vascular endothelial cells, predominantly in the hypothalamus, pituitary gland, hippocampus, and cerebral cortex (47). IL-1β is produced in the brain in response to central nervous system (CNS) and systemic infections (19, 25) and to ischemic (32, 45, 48) and traumatic brain injury (42), conditions that are associated with both fever and alterations in cerebral blood flow (CBF) (9, 16, 17).

Cerebral vasodilation after IL-1β injection has been shown to be mediated, in part, by the prostaglandins, 6-keto-PGF1α, PGE2, and PGF2α, through activation of cyclooxygenase-1 (COX-1) and COX-2 (23, 36, 41). These prostanoids are thought to cause early cerebral vasodilation by accumulation of cyclic nucleotides (4) and, later, by stimulation of the inducible form of nitric oxide (NO) synthase (iNOS) in vascular smooth muscle and endothelial cells, thereby increasing the production of NO (7, 14, 21).

In previous studies, the temporal relationship between the cerebrovascular and thermoregulatory effects of IL-1β would be attenuated by inhibiting the production of nitric oxide (NO). Adult male rats received 100 ng intracerebroventricular (icv) injection of IL-1β, and cortical CBF (cCBF) was measured by laser-Doppler in the contralateral cerebral cortex. A central injection of IL-1β caused a rapid increase in cCBF to 133 ± 12% of baseline within 15 min and to an average of 137 ± 12% for the remainder of the 3-h experiment. Brain and rectal temperature increased by 0.4 ± 0.2 and 0.5 ± 0.2°C, but not until 45 min after IL-1β administration. Pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME; 5 mg/kg iv) completely prevented the changes in cCBF and brain and rectal temperature induced by IL-1β. L-Arginine (150 mg/kg iv) partially reversed the effects of L-NAME and resulted in increases in both cCBF and temperature. These findings suggest that the vasodilatory effects of IL-1β in the cerebral vasculature are independent of temperature and that NO plays a major role in both the cerebrovascular and thermoregulatory effects of centrally administered IL-1β.

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l-NAME attenuates the changes in CBF and temperature after central administration of IL-1β.

METHODS

This study was approved by the University of Miami Animal Care and Use Committee. Young adult (2–3 mo) male Sprague-Dawley rats were anesthetized with 5% isoflurane-70% nitrous oxide (N2O), balance oxygen, and were endotracheally intubated and ventilated with a rodent respirator (model 50095–1, Stoelting; Wood Dale, IL) to achieve normal arterial blood gases. Anesthesia was maintained with 1–2% isoflurane-70% N2O, balance oxygen, and the rats were paralyzed with pancuronium (1 mg·kg⁻¹·h⁻¹ iv). The femoral vessels were catheterized for measurement of mean arterial pressure (MAP), blood sampling, and drug administration. A thermocouple probe was inserted into the rectum to a distance of 8 cm for continuous temperature monitoring, and then the animal was placed in a stereotaxic frame to immobilize the cranium. The dorsal scalp was reflected from 3 mm anterior to bregma to the posterior neck muscles. A 1-mm-diameter craniotomy was made 2 mm posterior and 3 mm lateral to bregma on the right, through which a 30-gauge thermocouple probe was inserted to a depth of 2 mm so that its tip would lie within the cerebral cortex. A second craniotomy was made on the right 0.8 mm posterior and 1.5 mm lateral to bregma for icv injection into the right lateral ventricle. Icv injections (10 μl) were made by using a micropipette at a depth of 4 mm into the right lateral ventricle over 2 min to prevent changes in intracranial pressure. On the left, a third craniotomy, measuring 2 × 3 mm, was made 2 mm posterior and 3 mm lateral to the bregma, exposing the intact dura. All of the craniotomies were made under saline irrigation down to a thin layer of bone, which was then excised with microforceps. To measure cCBF, a 0.8-mm-diameter laser-Doppler flow probe was positioned with a micromanipulator adjacent to the dura over an area free of large vessels in the lateral parietal cortex and connected to a tissue perfusion monitor (model ALF-21D, Transonic Systems; Ithaca, NY).

The arterial catheter was connected to a Statham pressure transducer (model 13–4615–50, Gould; Cleveland, OH), which was calibrated before each experiment. Measurements of arterial blood pressure and cCBF were recorded continuously (model RS3600, Gould). Arterial blood gases and pH, plasma glucose and lactate concentrations were measured hourly (model YSI 2300, Yellow Springs Instruments).

Brain and rectal temperatures were recorded every 15 min. After all of the surgical manipulations had been completed, isoflurane, but not N2O, was discontinued. The next 60 min were used to establish a stable baseline temperature and cCBF. During this time, rectal and brain temperatures were kept at 37.5 ± 0.3°C with the use of heating lamps. Once temperature stability was achieved, the position of the heating lamps was held constant for the remainder of the experiment.

The experiment was divided into two phases. The first phase was a prospective, blinded study with two experimental groups. Group I (n = 7) received 100 ng icv of heat-inactivated IL-1β (75°C for 2 h) in artificial CSF. Group II (n = 7) received 100 ng icv of recombinant rat IL-1β (Endogen; Woburn, MA) in artificial CSF. A single dose of IL-1β 100 ng icv was chosen because in pilot studies this dose consistently caused a significant and sustained increase in both CBF and rectal temperature, as opposed to 25 or 50 ng IL-1β. Groups I and II were monitored for 180 min after administration of IL-1β. In the second phase of the study, Group III (n = 8) received l-NAME 5 mg/kg iv 20 min before IL-1β (100 ng icv), and the responses were monitored for 105 min. At minute 105, a L-arginine 150 mg/kg iv bolus was given, followed by a continuous infusion 150 mg·kg⁻¹·h⁻¹ iv for 75 min to reverse the effects of l-NAME.

At the end of 3 h, the animals were euthanized with an overdose of anesthetic and intravenous potassium chloride. Immunohistochemistry for IL-1β was performed in four animals randomly selected from group II at 30, 45, and 60 min after icv injection of IL-1β, by using a polyclonal antibody to rat IL-1β (Endogen). Transcardiac perfusion-fixation of the brain was performed with ice-cold PBS (pH 7.4) for 1 min and ice-cold 4% paraformaldehyde (pH 7.4) for 5 min. The brains were quickly removed and placed in 4% paraformaldehyde at 4°C for 24 h and then transferred to PBS until sectioning. Coronal sections (50 μm) were obtained with the use of a tissue sectioning device (Vibratome; St. Louis, MO) at four levels from the anterior striatum to the anterior hippocampus. Sections were then washed, quenched with H2O2, and incubated with polyclonal rabbit anti-rat IL-1β antibody. They were washed again, incubated in diaminobenzidine tetrahydrochloride for staining, mounted on glass slides, and examined under light microscopy.

Measurements of brain and rectal temperature, MAP, and cortical CBF were averaged over 5 min for each time point. Data were analyzed by repeated-measures ANOVA with post hoc Bonferroni test. Values are expressed as means ± SE, with P < 0.05.

RESULTS

There were no significant differences between groups for MAP, cCBF, arterial blood gases, temperature, and plasma glucose and lactate at baseline, and PaCO2 was tightly controlled between 35–40 mmHg throughout the experiment in all groups. Fifteen minutes after icv injection of IL-1β, cCBF increased to 133 ± 12% of baseline in group II (P < 0.05). This increase in cCBF was sustained at an average of 137 ± 12% of baseline for the remainder of the 3-h experiment (Fig. 1). Heat-inactivated IL-1β caused no statistically significant changes in either cCBF or brain and rectal temperature. After IL-1β, brain and rectal temperature increased by 0.4 ± 0.2 and 0.5 ± 0.2°C, respectively, over baseline at 45 min after central in-
Injection of IL-1β and remained at an average of 0.7 ± 0.2°C and 0.9 ± 0.2°C, respectively, above baseline for the remainder of the experiment (Fig. 2). There was a strong correlation between brain and rectal temperature ($R^2 = 0.91$, $P < 0.01$) in all experimental groups, with an average rectal-brain difference of 0.3°C. The observed increase in cCBF preceded the observed increase in brain temperature by ~30 min (Fig. 3).

In group III, L-NAME increased MAP from 128 ± 1 to 156 ± 3 mmHg within 15 min (Fig. 4), lowered rectal temperature from 37.7 ± 0.08 to 37.5 ± 0.09 ($P < 0.01$), and did not alter brain temperature. Before IL-1β, L-NAME also reduced cCBF by 20 ± 4% ($P < 0.01$). In the presence of L-NAME, IL-1β had no effect on either cCBF or brain and rectal temperature. When L-arginine was given 105 min after central injection of IL-1β, MAP, cCBF, and brain and rectal temperature returned to baseline (Figs. 5 and 6).

Immunohistochemistry revealed IL-1β-positive cells in the superficial cerebral cortex and the periventricular surfaces (Fig. 7). This showed that after the icv injection, IL-1β was widely distributed over the brain cortex and periventricular region, with only superficial penetration at the time points studied. Specificity of the primary antibody was confirmed when no immunoreaction product was present after preadsorption with recombinant rat IL-1β at a molar ratio of 1:2.5.

**DISCUSSION**

These data show that the early increase in cCBF after central injection of IL-1β is independent of temperature and that NO plays a major role in both the cerebrovascular and thermoregulatory effects of IL-1β. This study is unique in that we measured these two important effects of IL-1β simultaneously in the same animals. Our findings support the idea that the pathways for the production of NO and prostaglandins in response to IL-1β are in series (4, 28, 36, 41) rather than in parallel (34).

The cerebrovascular effects of IL-1β have been studied in a variety of experimental paradigms. Direct application or superfusion of IL-1β onto cerebral arteries has demonstrated rapid increases in vessel diameter associated with elevations in CSF concentration of eicosanoids and cGMP (27, 41, 43). With the use of the closed cranial window technique on newborn piglets, Shibata et al. (41) found that IL-1β increased pial arteriolar diameter by 18.6% within 10 min, accompanied by increases in CSF concentrations of prostanoids, cAMP and cGMP. Osuka et al. (28) measured a 28% increase in basilar artery diameter 2 h after application of IL-1β. In both of these studies, the vasodilatory...
effects of IL-1β were prevented by pretreatment with COX inhibitors, suggesting a role for both prostanoids and NO in the vasodilation induced by IL-1β. Armstead (4) demonstrated that the vasodilatory effects of the prostaglandins PGI₂ and PGE₂ are partially dependent on NO production, because the NOS inhibitor L-NAME attenuated prostaglandin-induced vasodilation. Other investigators have shown that PGD₂ plays an important role in the rat’s response to IL-1β (44) and in preventing the upregulation of iNOS in vascular smooth muscle cells (17). In the present study, we measured a 40% increase in cCBF 15 min after central administration of IL-1β, which was completely prevented by L-NAME. Such a rapid increase in cCBF after IL-1β probably reflects activation of cNOS (5, 18, 26) rather than iNOS, because several studies (7, 21, 47) have shown that upregulation of the latter isoform of NOS does not occur until at least 6 h after administration of IL-1β. 

The thermoregulatory effects of IL-1β have been well studied; however, the role of NO in this response has not been clearly delineated [for review, see Schmid et al. (40)]. As is the case for IL-1β-induced vasodilation, PGE₂ has been thought to be the primary mediator of the febrile response to IL-1β (12, 13, 22, 35). More recently, NO has been found to play an important role in thermoregulation, although conflicting studies have shown both pyretic and antipyretic effects of NO. Almeida et al. (1) found that centrally injected l-NAME increased body temperature and enhanced the febrile response to LPS. In contrast, systemic administration of either l-NAME or the neuronal NOS inhibitor 7-nitroindazole lowers body temperature and inhibits the rise in temperature after LPS infusion (8, 34, 37). Although the systemic vasoconstrictive effects of l-NAME should decrease heat loss through the skin and thus elevate core temperature, we found that l-NAME decreased rectal temperature and prevented the febrile response to central administration of IL-1β. Roth et al. (33) reported similar results in rabbits that were given both IL-1β and l-NAME intraperitoneally, the latter at 10× the dose used in our study. In that study, no attempt was made to reverse the effects of l-NAME. When L-arginine was given 105 min after l-NAME in our study, both cCBF and temperature increased significantly to pre-l-NAME values but did not reach the levels seen in rats that received IL-1β alone. The incomplete reversal of the effects of l-NAME could have been due to an inadequate dose of L-arginine, although in pilot studies in rats in our lab and in infant piglets, complete reversal of the hemodynamic effects of l-NAME were achieved with L-arginine at 30× the dose of L-NAME (38). A more likely explanation is that insufficient IL-1β was present in CSF at the time of reversal with L-arginine, due to rapid clearance or metabolism of centrally administered IL-1β (12).

In the absence of l-NAME, IL-1β increased cCBF and, later, brain and body temperature. The delayed elevation in brain temperature did not further increase cCBF over the level induced initially by IL-1β. The apparent lack of additive effects of IL-1β and fever on cCBF could be explained by the following possibilities: 1) that IL-1β and fever increase cCBF by the same mechanism, e.g., through the production of NO, 2) that the increase in brain temperature was too small to
cause a change in CBF, and 3) that the laser-Doppler method of measuring CBF was not sensitive enough to detect a small change in CBF, which may have occurred when brain temperature increased after IL-1β was given. Alternatively, the vasodilatory effects of IL-1β may have been receding at the same time that the same effects of fever were beginning, yielding no change in CBF.

Another interesting issue relates to the separate roles of NO and prostaglandins in the effects of IL-1β on CBF and temperature. Assuming that both compounds are involved in these effects of IL-1β, the following questions arise concerning their synthetic pathways: 1) are they in series or in parallel; 2) are they convergent or divergent; 3) which is produced first and in greater amounts; and 4) are there cell-specific differences in the effect of IL-1β on these pathways? These questions require further investigation.

In conclusion, we found that the increases in both CBF and temperature in response to IL-1β were completely prevented by pretreatment with the nonselective NOS inhibitor, L-NAME, suggesting that NO plays an important role in these responses to IL-1β. Cortical CBF increased before hyperthermia occurred, indicating that the early cerebrovascular effects of IL-1β are independent of temperature. Our findings underscore the important role of NO in brain disorders such as meningitis (19, 25), stroke (20, 24, 32, 45, 48), and traumatic brain injury (42) in which inflammatory cytokines may augment the primary injury as well as cause disturbances in thermoregulation leading to fever. Whereas the cerebrovascular effects of NO are clearly vasodilatory in the presence of IL-1β, it appears that the thermoregulatory effects of NO depends upon the dose and species studied, the route of administration and the site and cell types in the brain involved in its production and action (40).

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