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Effects of a neuronal nitric oxide synthase inhibitor on lipopolysaccharide-induced fever

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Abstract

It has been demonstrated that nitric oxide (NO) has a thermoregulatory action, but very little is known about the mechanisms involved. In the present study we determined the effect of neuronal nitric oxide synthase (nNOS) inhibition on thermoregulation. We used 7-nitroindazole (7-NI, 1, 10 and 30 mg/kg body weight), a selective nNOS inhibitor, injected intraperitoneally into normothermic Wistar rats (200-250 g) and rats with fever induced by lipopolysaccharide (LPS) (100 µg/kg body weight) administration. It has been demonstrated that the effects of 30 mg/kg of 7-NI given intraperitoneally may inhibit 60% of nNOS activity in rats. In all experiments the colonic temperature of awake unrestrained rats was measured over a period of 5 h at 15-min intervals after intraperitoneal injection of 7-NI. We observed that the injection of 30 mg/kg of 7-NI induced a 1.5°C drop in body temperature, which was statistically significant 1 h after injection ($P<0.02$). The coinjection of LPS and 7-NI was followed by a significant ($P<0.02$) hypothermia about 0.5°C below baseline. These findings show that an nNOS isoform is required for thermoregulation and participates in the production of fever in rats.

Key words
- Nitric oxide
- 7-Nitroindazole
- Body temperature
- LPS
- L-NAME

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Introduction

Nitric oxide (NO) is synthesized from L-arginine via NO synthase (NOS) (1). At least three NOS isoforms exist: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (2,3). eNOS is largely found in endothelial cells and plays a substantial role in blood pressure control (1). nNOS is encountered in spinal cord, brain, kidney, and sympathetic ganglia (4), and most of the NO in the brain is believed to be synthesized by the action of neuronal NOS (4).

The relationship between NO production and thermoregulation has been studied recently. It has been shown that NO may act on vascular smooth muscle, decreasing vascular tone, stimulating thermogenesis in brown fat, and modulating neuroendocrine function (5-7).

Animals injected with lipopolysaccharide (LPS) produce cytokines including interleukins and tumor necrosis factor which may increase body temperature (8). The involvement of NO in thermoregulation is suggested by *in vivo* work showing that systemic inhibition of NOS during LPS injection acutely increases the bioactivity of both plasma interleukin and tumor necrosis factor (9), substances that elicit thermoregulatory and other

neural responses to endotoxemia. Furthermore, systemic administration of L-nitromonomethyl-L-arginine (L-NAME), an arginine analog, resulted in significant hypothermia and reduction of LPS fever (10). L-NAME is a nonspecific NOS inhibitor, whereas 7-nitroindazole (7-NI) is a selective nNOS inhibitor (11,12). It has been demonstrated that 30 mg/kg 7-NI given intraperitoneally may inhibit 60% of nNOS activity in rats (13). Therefore, the aim of the present study was to determine whether blockade of nNOS by 7-NI affects the body temperature of normothermic rats and rats with fever induced by lipopolysaccharide injection.

Material and Methods

Animals

Experiments were performed on adult male Wistar rats weighing 200-250 g, housed at controlled temperature (25 ± 2°C) and exposed to a daily 12:12-h light-dark cycle. The animals were allowed free access to water and food. Experiments were performed between 12:00 a.m. and 6:00 p.m. In all experiments body temperature was measured over a period of 5 h at 15-min intervals after intraperitoneal injection (0.5 ml). Body temperature was measured by inserting a thermoprobe into the colon. It should be pointed out that, before the experiment, the animals were habituated to temperature measurements which were performed quickly to avoid any stress-induced elevation in temperature.

Determination of the effect of L-NAME on normal body temperature

After an initial 30-min period, the rats were injected intraperitoneally with L-NAME (Sigma Chemical Co., St. Louis, MO, USA). Test animals received 1, 10, or 30 mg/kg L-NAME and control animals 0.9% saline and the effects on body temperature were determined.

Determination of the effect of 7-NI on normal body temperature

Control body temperature was determined after an initial 30-min period and rats were treated with 7-NI (Calbiochem Corporation, La Jolla, CA, USA), by intraperitoneal injection of 1, 10 or 30 mg/kg body weight 30 min before the body temperature measurements. 7-NI was dissolved in vehicle consisting of dimethyl sulfoxide/sesame oil (1:9). Control animals were treated with an intraperitoneal injection of the vehicle (dimethyl sulfoxide/sesame oil).

Determination of the effect of LPS injection on body temperature

The animals received LPS (Calbiochem-Novabiochem Corporation, La Jolla, CA, USA) dissolved in pyrogen-free sterile saline by intraperitoneal injection of 100 µg/kg body weight. Control animals received an intraperitoneal injection of saline (0.5 ml).

Determination of the combined effects of LPS and the nNOS inhibitor

To determine whether L-NAME and 7-NI influence the temperature increase produced by LPS, immediately after intraperitoneal injection of 100 µg/kg of LPS the rats received an intraperitoneal injection of 30 mg/kg 7-NI or 30 mg/kg L-NAME. Control animals were injected with the same volume of vehicle.

Statistical analysis

All data are reported as means ± SEM. For the statistical analysis we compared the mean change in body temperature in the test groups at 1, 2, 3, 4 and 5 h after injection, with the control group using an independent *t*-test without assuming equal variances in the two groups. Because all of the experi-

ments involve multiple comparisons to control groups, the difference between means was assessed by the Tukey-Kramer multiple-comparison test. Values of $P<0.05$ were considered to be significant.

Results

In all experiments, mean baseline body temperature ranged from 36.8° to 37.8°C, with no difference between experimental and control groups.

Effect of intraperitoneal injection of L-NAME

Figure 1 shows the effect of L-NAME (1, 10 and 30 mg/kg) injected intraperitoneally on body temperature. The injection of 1 mg/kg L-NAME did not significantly change body temperature. In contrast, 10 and 30 mg/kg of L-NAME significantly decreased ($P<0.05$) body temperature 1 h after injection in normothermic rats when compared to saline-injected rats.

Effect of intraperitoneal injection of 7-NI on normal body temperature

Figure 2 shows the effect of 7-NI on body temperature in normothermic rats. When 1 mg/kg of 7-NI was injected a nonsignificant decrease in body temperature was observed, while 10 and 30 mg/kg of 7-NI significantly decreased body temperature. The injection of 10 mg/kg of 7-NI produced a 0.5°C drop in body temperature, which was significantly lower than in the control group 30 min after injection ($P<0.05$). The injection of 30 mg/kg of 7-NI induced a 1.5°C drop in body temperature, which was statistically significant 1 h after injection ($P<0.02$).

Effect of combined intraperitoneal injection of LPS and L-NAME or LPS and 7-NI on body temperature

Figure 3 shows that the combination of vehicle injection and LPS (100 µg/kg) caused a significant increase in body temperature

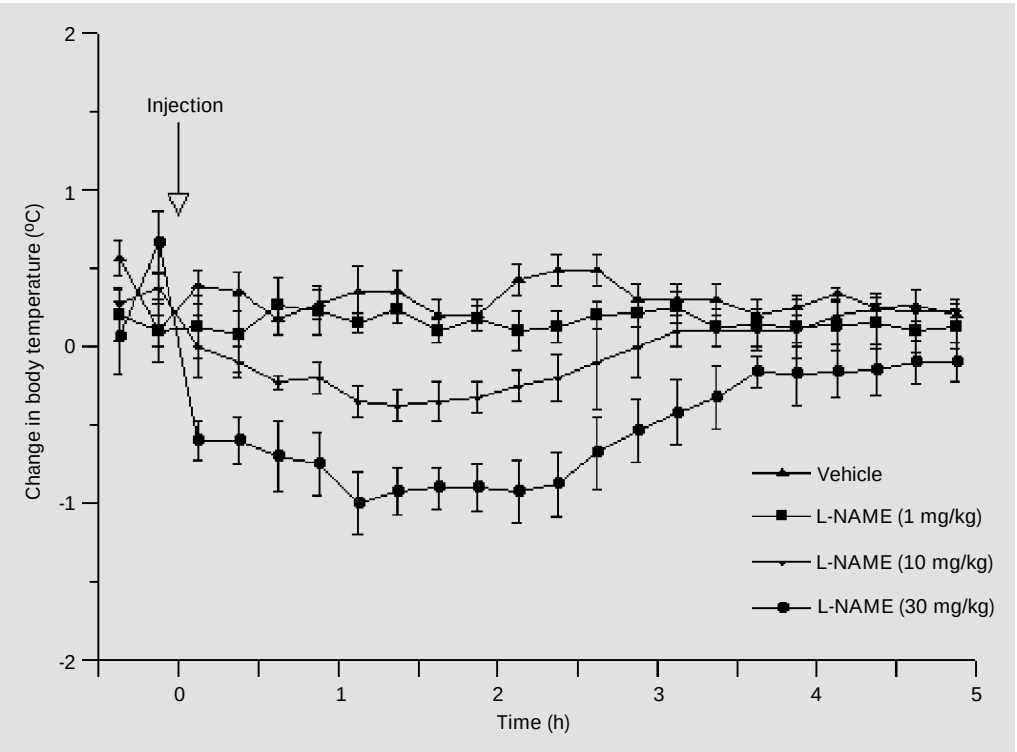

Figure 1 - Effect of intraperitoneal injection of N^G-nitro-L-arginine methyl ester (L-NAME) on body temperature. Data are reported as mean changes in temperature (± SEM) determined at 15-min intervals relative to baseline. N = 5 in each group.

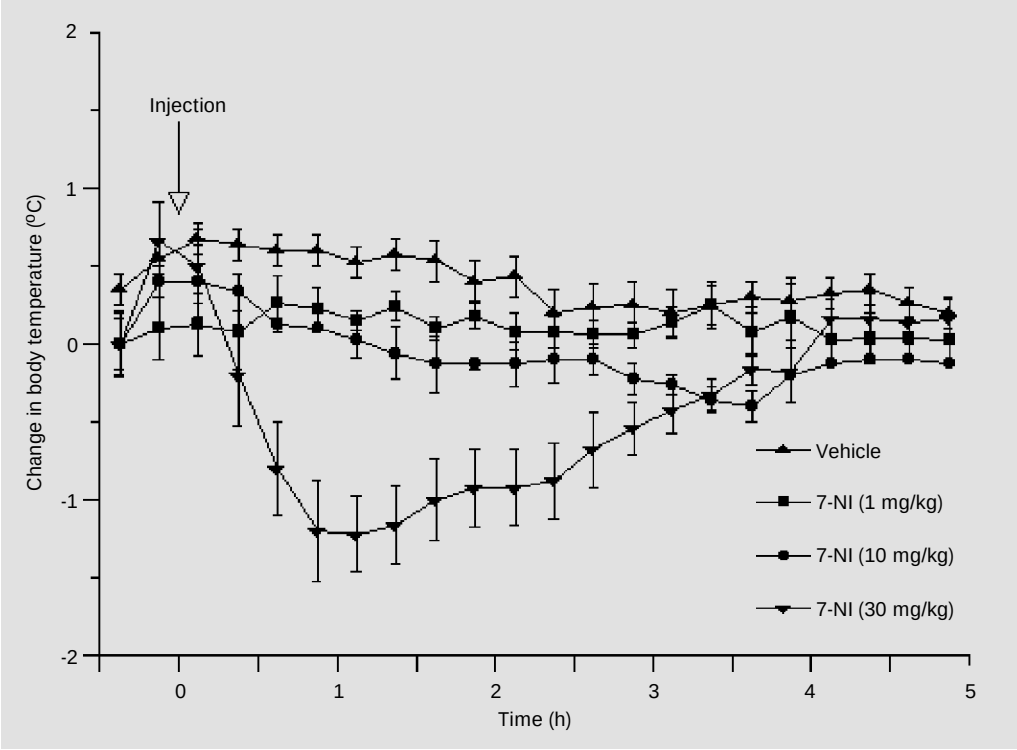

Figure 2 - Effect of intraperitoneal injection of 7-nitroindazole (7-NI) on body temperature. Data are reported as mean changes in temperature (± SEM) determined at 15-min intervals relative to baseline. N = 5 in each group.

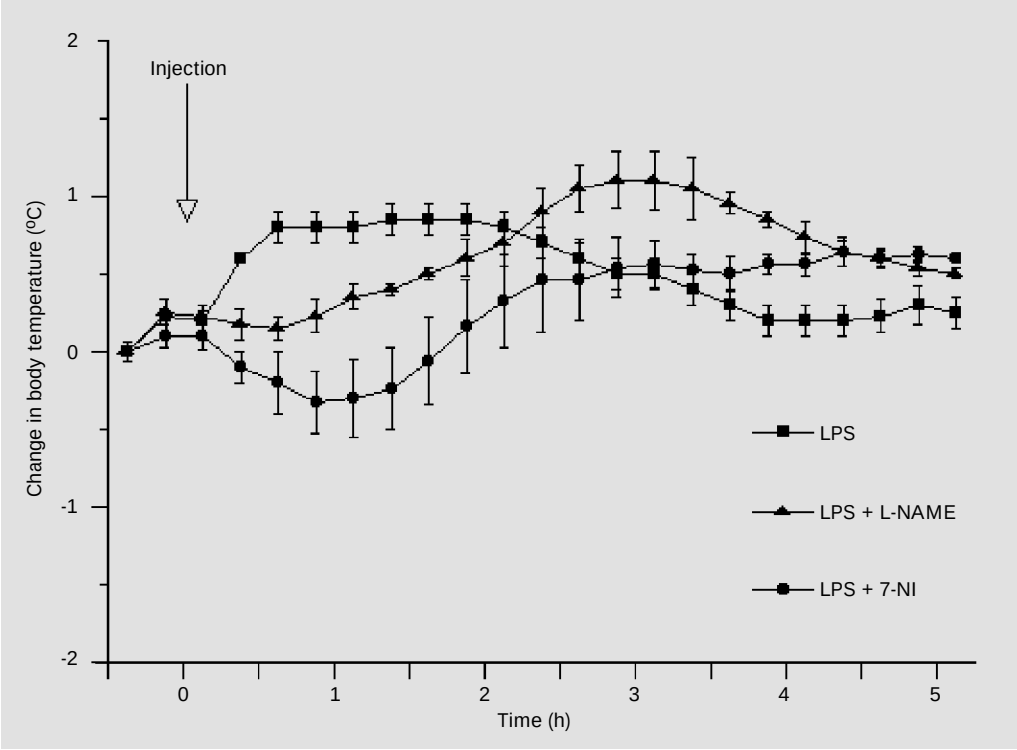

Figure 3 - Body temperature of rats injected with 100 µg/kg lipopolysaccharide (LPS) combined with 30 mg/kg N^G-nitro-L-arginine methyl ester (L-NAME) and with 30 mg/kg 7-nitroindazole (7-NI) compared to rats that received only LPS. Data are reported as mean changes in temperature (± SEM) determined at 15-min intervals relative to baseline. N = 5 in each group.

that persisted for 2 h after injection. When L-NAME was co-injected intraperitoneally with LPS the body temperature significantly dropped to basal levels up to the third hour, when it started to increase again ($P<0.04$), returning to basal levels within 5 h. To determine whether 7-NI influenced the increase in body temperature induced by intraperitoneal injection of LPS, we coinjected 30 mg/kg of 7-NI with 100 µg/kg of LPS. The coinjection of LPS and 7-NI was followed by a significant ($P<0.02$) hypothermia about 0.5°C below baseline that was followed by a more gradual return to baseline.

Figure 4 shows the comparison between LPS and 7-NI to determine if 7-NI affects fever and between LPS/L-NAME and LPS/7-NI to determine if the effects of blocking nNOS differ from nonspecific blockade, at the second (Figure 4A) and fourth (Figure 4B) hour after the injection. We found that 7-NI induced a significant decrease in LPS-induced fever at the second hour (Figure 4A), but after the fourth hour the body temperature was almost the same when compared to rats injected with LPS/vehicle. When we compared the effect of LPS/L-NAME to that of LPS/7-NI we found that 7-NI was more effective than L-NAME in decreasing body fever induced by LPS injection.

Discussion

In the present study we investigated the effects of nNOS inhibition on the body temperature of conscious rats with the use of the relatively selective nNOS inhibitor 7-NI. The new findings of the present study are: 1) blockade of nNOS decreased the body temperature of normothermic rats inducing a dose-dependent hypothermia, and 2) 7-NI abolished the increase in body temperature induced by intraperitoneal injection of LPS.

A number of recent studies have shown that NO accounts for a large part of the biological actions of endothelium-derived relaxing factor (14) and the importance of NO has been demonstrated by inhibition of NOS (15). Recently, Scammell et al. (10) showed that L-NAME, a nonspecific NOS inhibitor, reduces the febrile response to LPS and suggested that NO plays a role in normal thermoregulation and is required for the production of fever. Our study adds the participation of the nNOS isoform in body temperature control by the effect of systemic administration of 7-NI. In the group of the

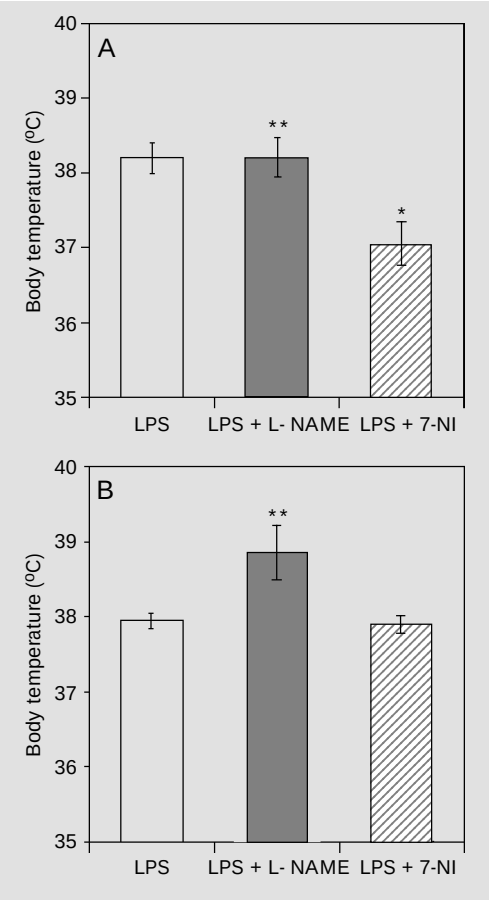

Figure 4 - Body temperature of rats injected with 100 µg/kg lipopolysaccharide (LPS) combined with 30 mg/kg N^G-nitro-L-arginine methyl ester (L-NAME) and with 30 mg/kg 7-nitroindazole (7-NI) compared to rats that received only LPS. Comparison at a single time; second (A) and fourth (B) hour after the nitric oxide inhibitor or vehicle injection. Data are reported as mean body temperature (± SEM). *P<0.05 LPS compared to LPS/7-NI and **P<0.05 LPS/L-NAME compared to LPS/7-NI (Tukey-Kramer multiple comparison test). N = 5 in each group.

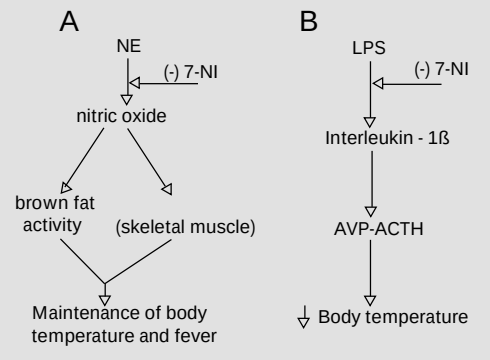

Figure 5 - Possible mechanism through which the inhibition of nNOS could influence body temperature under normothermic conditions and during fever induced by LPS. Nitric oxide increases glucose transport in skeletal muscle and body temperature. 7-Nitroindazole (7-NI) induces hypothermia (A) and abolishes the febrile response by inhibiting nNOS (B). ACTH, Adenocorticotropin; AVP, vasopressin; NE, norepinephrine; (-), inhibition of nitric oxide synthesis.

imidazoles and indazoles, 7-NI has been used as a selective inhibitor of nNOS (13).

A few technical aspects of these experiments deserve comment. Although we did not measure NOS activity after the use of the nNOS blocker, others have reported the effects of 30 mg/kg 7-NI given intraperitoneally on nNOS were maximal within 30 min, and NOS activity continued to be 60% inhibited over 3 h and returned to basal values after 4 to 24 h (13).

The mechanisms involved in the modulation of body temperature control are complex. Our finding was that the hypothermia induced by the effects of 7-NI administration may reflect the results of nNOS inhibition at different sites, including brown adipose tissue, vascular smooth muscle, skeletal muscle activity, or central regulation of body temperature. A mechanism that can promote a decrease in body temperature is by impairment of brown fat thermogenesis (10). Sympathetic stimulation increases blood flow through brown fat and triggers nonshivering thermogenesis (16). Recently it has been demonstrated that nNOS is found mostly in sympathetic ganglia (4), and intravenous injection of L-NAME blocks the norepinephrine-induced rise in brown fat as well as the increase in brown fat blood flow (1) (Figure 5). A secondary mechanism that may alter body temperature is the increase in blood flow to superficial vascular beds which may induce heat loss. However, it has been demonstrated that 7-NI has minimal pressor effects *in vivo* (17).

Recent immunocytochemical studies (18, 19) have reported that both nNOS and eNOS isoforms are expressed in skeletal muscle. Together with these findings, it has been demonstrated that NO is released from incubated skeletal muscle preparations (20). Although the complete physiological significance of NO in skeletal muscle remains to be demonstrated, a number of different laboratories (18,21,22) have noted that NO may play a role by increasing contractile function. In addition, it has also been shown that nitric oxide increases glucose transport in skeletal muscle (23). It is well established that skeletal muscle activity is closely related to body temperature control in both normothermic and hyperthermic conditions. Thus, we suggest that nNOS inhibition could induce hypothermia by decreasing the heat production from skeletal muscle (Figure 5A).

The sites of action of NO produced by NOS seem to be distributed throughout the body, including the central nervous system, where they may influence thermoregulation (24). However, a previous study from our laboratory showed that intracerebroventricular injection of L-NAME induced an increase in body temperature (25).

In the present experiment, the administration of 7-NI almost completely blocked the increase in body temperature induced by LPS. This finding could be explained by the same mechanisms that cause the decrease in baseline temperature. In addition, it has been demonstrated that NO may participate in the release of hypothalamic hormones that induce a decrease in body temperature. L-NAME potentiates the release of vasopressin and adrenocorticotropin induced by interleukin 1ß and LPS (7,26). Thus, during LPS-induced fever nNOS inhibition could result in an increased release of these two peptides.

In the present investigation we also studied the effects of L-NAME on rats with LPS-induced fever. The relatively selective nNOS inhibitor 7-NI was more effective than L-NAME in inducing hypothermia in rats after LPS-induced fever (Figure 4A). These findings highlight the importance of nNOS in body temperature control and suggest that nNOS may be the primary NOS isoform that participates in the thermoregulation and production of fever induced by LPS.

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