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Role of nitric oxide in systemic effect of theophylline on mouse body temperature

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Abstract

In the present study, the interaction of nitric oxide synthase (NOS) inhibitors, L-NAME (N^G-nitro-L-arginine methyl ester HCl) and L-NA (N^ω-nitro-L-arginine), and its precursor, L-arginine (2-(*S*)-2-amino-5-[(aminoiminomethyl)amino] pentatonic acid), with theophylline on mouse body temperature was studied. Intraperitoneal (i.p.) injection of different doses of theophylline altered body temperature. Lower doses of theophylline (12.5 and 25 mg/kg) increased, but a higher dose (100 mg/kg) reduced, the animals' body temperature. The combination of L-arginine (20 and 40 mg/kg) with the highest dose of theophylline potentiated the hypothermic effect induced by the latter drug, while L-arginine by itself did not alter body temperature. L-NAME (10-80 mg/kg) or L-NA (10 mg/kg) plus a lower dose of theophylline (12.5 mg/kg) reduced the theophylline-induced hyperthermic response. L-NA (1, 5, and 10 mg/kg) in combination with the high dose of theophylline (100 mg/kg) also induced greater hypothermia. Both L-NAME and L-NA by themselves reduced body temperature. It is concluded that nitric oxide (NO) may be involved in the effects of theophylline on body temperature in mice.

Keywords: Theophylline; Nitric oxide (NO); L-Arginine; N^G-nitro-L-arginine methylester (L-NAME); N^ω-nitro-L-arginine (L-NA); Temperature (mouse)

1. Introduction

Nitric oxide (NO), which is enzymatically formed from L-arginine (2-(*S*)-2-amino-5-[(aminoiminomethyl)amino] pentatonic acid) by nitric oxide synthase (NOS) (Palmer et al., 1988), functions as an important intracellular mediator in the central nervous system (Garthwaite, 1991; Garthwaite et al., 1988; Knowles et al., 1994). It has been proposed that NO may play a role in the regulation of body temperature (Branco et al., 1997; Scammell et al., 1996). NO mediates a wide variety of physiological processes including relaxation of vascular smooth muscle, increase in brown fat thermogenesis, and alteration of neuroendocrine function (Ignarro et al., 1987; Nagashima et al., 1994; Rivier and Shen, 1994), which may influence thermoregulation. However, the exact mechanism of NO in thermoregulation is unknown. Furthermore, as shown in rat striatal tissue and in cultured rat fetal dopaminergic neurons, there is evidence that NO is implicated in the control of dopaminergic neurotransmission (Hanbauer et al., 1992; Zhu and Luo, 1992; Guevara-Guzman et al., 1994; Pogun et al., 1994).

Moreover, we have previously shown that theophylline interacts with modulatory mechanisms involved in thermoregulation. The hyperthermic and hypothermic effects of theophylline may be mediated through different systems including dopaminergic and cholinergic mechanisms (Zarrindast and Heidari, 1994). In the present study, interactions between theophylline and NO agents were evaluated.

2. Materials and methods

2.1. Animals

Male NMRI mice (weight range 20–30 g) were used in these experiments. They remained in groups of 10 in their cages (45×30×15 cm^3) under a temperature of 24±2 °C on a 12-h light–dark cycle. Mice had free access to food and water. The animals were deprived of food 18 h before experiments, but water was available at all times. Each animal was used once only and was euthanized immediately after the experiments. Eight animals were used in each experiment.

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2.2. Drugs

The following drugs were used: theophylline (Sigma, UK), 2-(*S*)-2-amino-5-[(aminoiminomethyl)amino]pentatonic acid (L-arginine), N^G-nitro-L-arginine methyl ester HCl (L-NAME), and N^{ω}-nitro-L-arginine (L-NA; Tocris Cookson, UK). The drugs were dissolved in saline except for L-NA, which was dissolved in a drop of glacial acetic acid and then diluted with saline. The drugs were injected intraperitoneally (i.p.) in a volume of 10 ml/kg.

2.3. Drug treatment

2.3.1. Experiment 1

Different doses of theophylline (12.5, 25, 50, and 100 mg/kg, i.p.) were administered to mice. Body temperature was recorded 15, 30, 45, 60, 90, and 120 min after the drug injection.

2.3.2. Experiment 2

Animals were administered L-arginine (20, 40, and 80 mg/kg, i.p.), L-NAME (5, 10, 20, and 80 mg/kg, i.p.), or L-NA (1, 5, and 10 mg/kg, i.p.), and body temperature was recorded as in Experiment 1.

2.3.3. Experiment 3

Animals were injected with saline (10 ml/kg) or L-arginine (20, 40, and 80 mg/kg, i.p.) 60 min before either saline or theophylline administration (12.5 and 100 mg/kg). Body temperature was recorded after theophylline injection, for a period of 120 min.

2.3.4. Experiment 4

Animals were injected with saline (10 ml/kg) or L-NAME (10, 20, and 80 mg/kg, i.p.) 60 min before either saline or theophylline administration (12.5 and 100 mg/kg). Body temperature was recorded after theophylline injection, for a period 120 min.

2.4. Temperature recording

On the day of the experiment, mice were housed individually in experimental cages and allowed to rest for 1 h before drug injection. During this period, body temperature was measured at 10-min intervals in order to exclude the effect of handling on animal temperature. The core body temperature was recorded with a rectal thermistor probe (YSI 400, USA). The probe was lubricated with petroleum jelly before being inserted to a depth of 2 cm. Body temperature was recorded immediately before and 15, 30, 45, 60, 90, and 120 min after the drug injection, and the AUC (area under curve) between 15 and 120 min was calculated. The experiments took place between 0800 and 1400 h. The experimental protocol was approved by the Research and Ethics Committee of the School of Pharmacy, Tehran University of Medical Sciences (No. P-813/2000).

2.5. Statistical analysis

Comparisons between groups were made with one- or two-way analysis of variance (ANOVA) following Newman–Keul's test. A difference with $P<0.05$ between experimental groups was considered statistically significant.

3. Results

3.1. Effect of theophylline, NO precursor, and NOS inhibitors on mouse body temperature

Fig. 1 indicates the time-course effects of theophylline on body temperature. The drug was administered intraperitoneally (i.p.) and body temperature was recorded immediately before and 15, 30, 45, 60, 90, and 120 min after drug injection, and the AUC was calculated. Comparison of the AUCs of the drugs with that of control showed that theophylline (12.5, 25, 50, and 100 mg/kg) [one-way ANOVA, $F(4,35)=48.0$, $P<0.0001$] altered body temperature. Analysis indicated that the lower doses of theophylline, 12.5 mg/kg (AUC=4619±17, $P<0.001$) and 25 mg/kg (AUC=4583±17, $P<0.001$), induced hyperthermia, while the medium dose, 50 mg/kg (AUC=4478±19, $P>0.05$), did not alter body temperature and the highest dose of the drug, 100 mg/kg (AUC=4296±22, $P<0.001$), decreased body temperature as compared with that of the saline control group (AUC=4484±14).

Fig. 2 shows the effects of NO agents on the body temperature of mice. L-Arginine (Fig. 2A), 20 mg/kg (AUC=4504.3±13.3, $P>0.05$), 40 mg/kg (AUC=4385.7±32.1, $P>0.05$), and 80 mg/kg (AUC=4439.1±39.9, $P>0.05$), did not alter the body temperature of animals

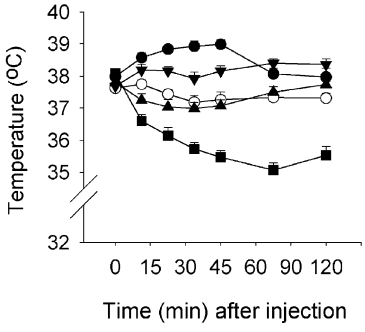

Fig. 1. Effect of theophylline on mouse body temperature. Mice were injected (i.p.) with saline (○) or theophylline (●) 12.5, (▼) 25, (▲) 50, and (■) 100 mg/kg. Hypothermia induced by the drug was recorded 15, 30, 45, 60, 90, and 120 min after drug administration. Each point is the mean±S.E.M. for body temperature of eight mice. The lower doses of the drug (12.5 and 25 mg/kg) increased, while the highest dose (100 mg/kg) decreased mouse body temperature (F values are in the Results section).

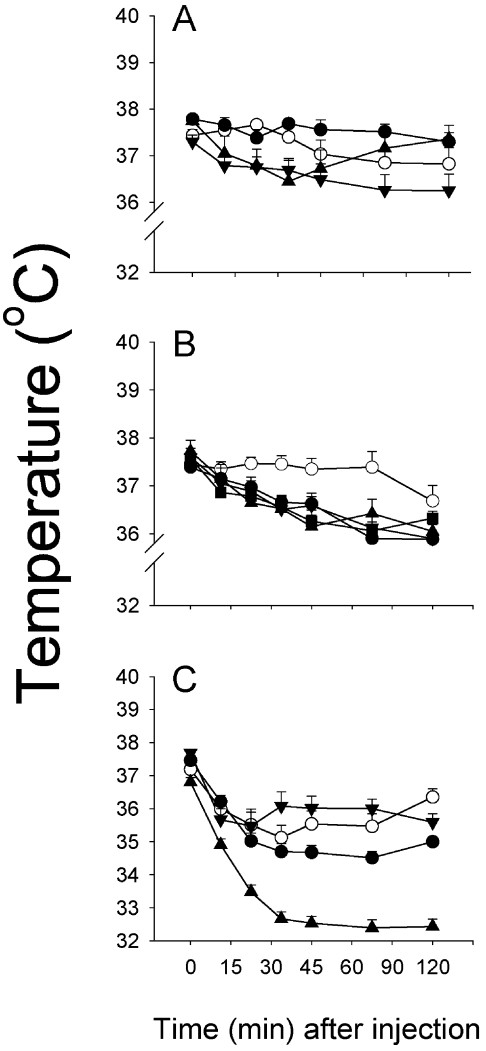

Fig. 2. Effect of NO precursor and inhibitors on mouse body temperature. Mice were injected (i.p.) with saline (○); (A) L-arginine (●) 20, (▼) 40, and (▲) 80 mg/kg; (B) L-NAME (●) 5, (▼) 10, (▲) 20, and (■) 80 mg/kg; or (C) L-NA (●) 1, (▼) 5, and (▲) 10 mg/kg. Hypothermia induced by the drugs was recorded 15, 30, 45, 60, 90, and 120 min after drug administration. Each point is the mean±S.E.M. for body temperature of eight mice. AUCs and *F* values for the drugs' effect are given in the Results section.

as compared with saline control group (AUC=4461.2±23.5) [one-way ANOVA, $F(3,28)=2.9$, $P>0.05$].

L-NAME (Fig. 2B), 5 mg/kg (AUC=4383.1±15.5, $P<0.05$), 10 mg/kg (AUC=4384.6±25.1, $P<0.05$), 20 mg/kg (AUC=4384.7±22.4, $P<0.05$), and 80 mg/kg (4377.7±20.7, $P<0.05$), decreased body temperature as compared with that of the saline group (AUC=4477.1±24.3) 24.3) [one-way ANOVA, $F(4,35)=3.8$, $P<0.05$]. A higher dose of L-NA (Fig. 2C), 10 mg/kg (AUC=3982.5±21.7, $P<0.001$) but not 1 mg/kg (AUC=4210.6±20.8, $P>0.05$) and 5 mg/kg (AUC=4314.9±36.3, $P>0.05$), decreased body temperature as compared with that of the saline control group (AUC=4287±38.6) [one-way ANOVA, $F(3,28)=24.5$, $P<0.0001$].

Table 1
Effect of L-arginine in the presence or absence of theophylline on the body temperature of mice

Drug	Saline	Theophylline (12.5 mg/kg)	Theophylline (100 mg/kg)
Saline	4378.4±22.3	4516.8±13.9[a]	4297.2±21.8[b]
L-Arginine (20 mg/kg)	4424.8±11.2	4512.6±31.3	4166.4±43.1[c]
L-Arginine (40 mg/kg)	4399.8±51.2	4427.7±62.6	4170.9±36.1[c]
L-Arginine (80 mg/kg)	4325.3±17.3	4407.8±29.7	4238.4±23.7

Mice were injected (i.p.) with saline and theophylline (12.5 and 100 mg/kg) alone or plus L-arginine (20, 40, and 80 mg/kg). L-arginine was administered 60 min prior to theophylline. Body temperature was recorded 15, 30, 45, 60, 90, and 120 min after theophylline administration. Each point is the mean±S.E.M. of AUCs for eight mice.

[a] $P<0.001$, different from saline control group.
[b] $P<0.01$, different from saline control group.
[c] $P<0.05$, different from the respective theophylline group.

3.2. Effects of NO precursor and NOS inhibitors on the effect of theophylline on mouse body temperature

Table 1 shows the effect of theophylline with or without L-arginine. The precursor of NO, L-arginine, was injected 60 min before theophylline. Body temperature was measured immediately before and 15, 30, 45, 60, 90, and 120 min after theophylline administration. The AUCs for change in temperature were calculated and are presented in the tables. Two-way ANOVA indicated that the effect of the adenosine receptor antagonist, theophylline (12.5 and 100 mg/kg), interacted with that of L-arginine (20, 40, and 80 mg/kg) [theophylline, $F(2,84)=57.1$, $P<0.0001$; L-arginine, $F(3,84)=3.1$, $P<0.05$; Theophylline×L-Arginine, $F(6,84)=2.4$, $P<0.05$]. Further analysis showed that L-arginine did not alter body temperature. The doses of 12.5 and 100 mg/kg of theophylline increased and decreased body temperature, respectively, and L-arginine elicited hypothermia in combination with the highest dose (100 mg/kg) of theophylline.

Table 2 shows the effect of theophylline with or without L-NAME. Two-way ANOVA showed that the effect of

Table 2
Effect of L-NAME in the presence or absence of theophylline on the body temperature of mice

Drug	Saline	Theophylline (12.5 mg/kg)	Theophylline (100 mg/kg)
Saline	4477.1±24.3	4526.5±21.3	4255.7±29.1[a]
L-NAME (10 mg/kg)	4324.3±29.7[b]	4448.8±11.7[c]	4274.9±30.5
L-NAME (20 mg/kg)	4384.7±22.4[c]	4459.9±16.3[c]	4350.4±26.3
L-NAME (80 mg/kg)	4256.3±33.6[d]	4358.4±20.7[d]	4322.4±42.5

Mice were injected (i.p.) with saline and theophylline (12.5 and 100 mg/kg) alone or plus L-NAME (10, 20, and 80 mg/kg). L-NAME was administered 60 min before theophylline. Body temperature was recorded 15, 30, 45, 60, 90, and 120 min after theophylline administration. Each point is the mean±S.E.M. of AUCs for eight mice.

[a] $P<0.001$, different from saline control group.
[b] $P<0.01$, different from respective theophylline group.
[c] $P<0.05$, different from respective theophylline group.
[d] $P<0.001$, different from respective theophylline group.

Table 3
Effect of L-NA in the presence or absence of theophylline on the body temperature of mice

Drug	Saline	Theophylline (12.5 mg/kg)	Theophylline (100 mg/kg)
Vehicle	4262.7±44.9	4384.5±14.0[a]	4358.1±16.7[a]
L-NA (1 mg/kg)	4156.5±71.5	4301.5±48.8	4203.0±26.4[b]
L-NA (5 mg/kg)	4139.7±44.0	4286.8±28.4	4098.6±29.2[c]
L-NA (10 mg/kg)	4050.2±41.4[d]	4217.7±49.2[d]	4071.8±51.0[c]

Mice were injected (i.p.) with saline and theophylline (12.5 and 100 mg/kg) alone or plus L-NA (1, 5, and 10 mg/kg). L-NA was injected 60 min prior to theophylline administration. Body temperature was recorded 15, 30, 45, 60, 90, and 120 min after theophylline administration. Each point is the mean±S.E.M. of AUCs for eight mice.

[a] $P<0.05$, different from saline control group.
[b] $P<0.01$, different from respective theophylline group.
[c] $P<0.001$, different from respective theophylline group.
[d] $P<0.05$, different from respective theophylline group.

theophylline (12.5 and 100 mg/kg) interacted with that of the NOS inhibitor, L-NAME (10, 20, and 80 mg/kg) [theophylline, $F(2,84)=30.0$, $P<0.0001$; L-NAME, $F(3,84)=9.5$, $P<0.0001$; Theophylline×L-NAME, $F(6,84)=5.8$, $P<0.0001$]. Further analysis showed that L-NAME reduced the mice's body temperature. The combination of L-NAME with the lower dose of theophylline (12.5 mg/kg) reduced the theophylline-induced hyperthermia. Moreover, L-NAME had no influence on the hypothermic response induced by the highest dose of theophylline (100 mg/kg).

Table 3 shows the effect of theophylline with or without L-NA. Two-way ANOVA shows that the AUCs of the effect of theophylline (12.5 and 100 mg/kg) did not interact with that of the inhibitor of NOS, L-NA (1, 5, and 10 mg/kg) [theophylline, $F(2,84)=13.8$, $P<0.0001$; L-NA, $F(3,84)=15.7$, $P<0.0001$; Theophylline×L-NA, $F(6,84)=0.8$, $P>0.05$]. However, further analysis showed that a 10-mg dose of L-NA reduced body temperature. The drug in combination with the lowest dose of theophylline (12.5 mg/kg) elicited a hypothermic response. L-NA in combination with the highest dose (100 mg/kg) induced even greater hypothermia, which may have been due to L-NA by itself.

4. Discussion

The present data show that administration of different doses of theophylline to mice altered their body temperature. The lower doses of theophylline increased, while the highest dose of the drug reduced, the animals' body temperature. Our previous study also showed a similar response to theophylline (Zarrindast and Heidari, 1994). Adenosine has been suggested to be an important mediator of hypoxia-induced hypothermia (Branco et al., 2000). Therefore, the hyperthermic response to theophylline, which is an adenosine receptor antagonist (Bruns et al., 1986), may be due to such a mechanism. Adenosine has also been shown to elicit hypothermia through two different A1 and A2 adenosine receptor subtypes (Zarrindast and Heidari, 1993). Moreover, theophylline has been shown to interact with modulatory mechanisms involved in thermoregulation. The hypothermia induced by the drug may be mediated through dopaminergic, cholinergic, and serotonergic mechanisms, while dopaminergic, cholinergic, and adrenergic systems may be involved in the hyperthermic effect of the drug (Zarrindast and Heidari, 1994). Theophylline is also a phosphodiesterase inhibitor at high doses (Choi et al., 1988). This mechanism may be involved in the hypothermic response to theophylline. The role of phosphodiesterase inhibition in the hypothermic action of methylxanthines proposed by others (Durcan and Morgan, 1991, 1992) might support this hypothesis.

Nitric oxide has been shown to be involved in systemic vasopressin-induced hypothermia and hypoxia-induced hypothermia in rats (Steiner et al., 1998; Branco et al., 1997). It has also been shown that inhibition of NOS produces hypothermia and depresses lipopolysaccharide-induced fever (Scammell et al., 1996). In the present study, interactions between NO agents and theophylline were evaluated. However, the precursor of NO, L-arginine, did not influence body temperature by itself, but the drug potentiated the hypothermia induced by the highest dose of theophylline. This may indicate that NO may be involved in the increase in theophylline-induced hypothermia.

Cyclic AMP (cAMP) signal transduction may play a role in the regulation of the endothelial production of NO (Zhang and Hintze, 2001), and considering the increase in cAMP levels induced by theophylline, the involvement of this mechanism in the potentiation of hypothermia induced by the combination of theophylline with L-arginine should be clarified.

It has been shown that dopamine may stimulate NOS and cause NO release through dopamine D1 receptors and, in contrast, D2 receptor stimulation may suppress NOS (Morris et al., 1997). Furthermore, both postsynaptic dopamine D1 and D2 receptors have been shown to be involved in hypothermia (Zarrindast and Tabatabai, 1992). Theophylline may also elicit the release of dopamine (Lin et al., 1980), and therefore induce hypothermia through dopamine receptors (Zarrindast and Heidari, 1994). Moreover, NO has been proposed to inhibit dopamine uptake (Pogun et al., 1994) and to increase dopamine release in the striatum (Liang and Kaufman, 1998). Therefore, the possibility exists that the potentiation of hypothermia by the combination of L-arginine with theophylline may be mediated by an interaction between dopamine receptor mechanisms and the NO system. However, other reports show that NO inhibits dopamine release in the rat hypothalamus (Seilicovich et al., 1995). Both theophylline and NO increase cGMP levels (Rosenzweig et al., 1999), and cGMP may increase cAMP (Kurtz et al., 1998), which may account for the potentiation of hypothermia induced by the combination of theophylline and L-arginine. Our data also showed that the NOS inhibitors, L-NAME and L-NA, by themselves reduced the body temper-

ature of the animals. The results are in agreement with those obtained by others (Scammell et al., 1996; Branco et al., 1997; Steiner et al., 1998) showing that NOS inhibitors are able to reduce body temperature. L-NAME has also been proposed to reduce food intake and oxygen consumption (DeLuca et al., 1995), which may account for the decrease in body temperature. Furthermore, our data indicate that the NO inhibitors interact with theophylline, and the combination of theophylline with L-NAME or L-NA induced a greater hypothermic response. This increase in hypothermia may be due to additive effects of the drugs.

It has been shown that NOS inhibitors increase cAMP levels in all areas of the central nervous system (Sidorov et al., 1999). These drugs have also been shown to elicit an increase in extracellular dopamine concentrations (Segieth et al., 2000). Furthermore, as we mentioned before theophylline also can increase dopamine release directly (Lin et al., 1980) or by inhibition of A1 adenosine receptors (Wood et al., 1989). Thus, the increase in the second messenger or dopamine may account for the hypothermia induced by L-NAME or L-NA and also for the potentiation of theophylline-induced hypothermia by these drugs. Moreover, it has been shown that both L-arginine and L-NAME inhibit the activity of several apoenzymes of P450, and thus decrease the systemic clearance of theophylline in rabbits (Barakat et al., 1997). Therefore, NO agents may increase the concentration of theophylline. This mechanism may be involved in the increase in theophylline-induced hypothermia elicited by NO agents.

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