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The Hyperthermic Effect of Nitric Oxide in Central Nervous System

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The precise mechanism of set-point regulation in hypothalamus was not elucidated. Nitric oxide synthases (NOS) were detected in hypothalamus, however, the roles of NO in hypothalamus was not fully studied. So, we tested the effects of NO on body temperature because preoptic-anterior hypothalamus was known as the presumptive primary fever-producing site. NO donor sodium nitroprusside (SNP, 4 nmol, i.c.v.) elicited marked febrile response, and this febrile response was completely blocked by indomethacin (a cyclooxygenase inhibitor). But, ODQ (selective guanylate cyclase inhibitor, 50 μg, i.c.v.) did not inhibit fever induced by SNP. The cyclic GMP analogue dibutyryl-cGMP (100 μg, i.c.v.) induced significant pyreses, which is blocked by indomethacin. N^G-nitro-L-arginine methyl ester (L-NAME, non selective NOS inhibitor) inhibited fever induced by interleukin-1 β (IL-1 β, 10 ng, i.c.v.), one of endogenous pyrogens. These results indicate that NO may have an important role, not related to stimulation of soluble guanylate cyclase, in the signal pathway of thermoregulation in hypothalamus.

Key Words: Nitric oxide, Nitroprusside, Pyrexia, i.c.v. injection

INTRODUCTION

It is widely accepted that inflammation and exogenous pyrogens (e.g., bacterial endotoxin) evoke fever through the activation of macrophages to release endogenous pyrogenic cytokines (IL-1 β, IL-6 and TNF-α) and the ending step in febrile response is the action of PGE_2 on thermoregulatory pathways in the hypothalamus (Coceani, 1990; Dinarello, 1990). Since the structural impermeability of cerebral capillaries and the low efficiency of any transport system for the cytokines across the capillary wall (Banks et al, 1991; Luheshi et al, 1994), it was proposed that circulating pyrogenic cytokines have their major effect on the rich vascular network close to the neurons in preoptic/anterior hypothalamus (i.e., organum vasculosum laminae terminalis [OVLT]) (Stitt, 1986). However, the precise mechanism by which blood-borne cytokines increase PGE_2 synthesis in hypothalamus is still unclear.

The nitric oxide (NO) mediates a wide variety of physiological processes that influence thermoregulation. NO relaxes vascular smooth muscle, increases brown fat thermogenesis, and influences neuroendocrine function (Ignarro et al, 1987; Nagashima et al, 1994; Rivier & Shen, 1994). These observations suggest that NO plays an important role in thermoregulation; however, it is currently unknown whether the centrally produced NO is a mediator in temperature regulation. Thus, we have investigated whether the modulation of NO production affect the development of pyrogenic fever in rats.

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METHODS

Chemicals

Interleukin-1 β (IL-1 β), sodium nitroprusside (SNP), N^G-nitro-$_L$-arginine methyl ester hydrochloride (L-NAME), dibutyryl-cGMP, 1H-[1,2,4]oxadiazolo-[4,3-a] quinoxalin-1-one (ODQ), and indomethacin were purchased from Sigma. IL-1 β, SNP and dibutyryl-cGMP were dissolved in artificial CSF solution (aCSF: 138 mM NaCl, 50 mM KCl, 11 mM $NaHCO_3$, 1 mM

KH_2PO_4, 1.1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4). ODQ was dissolved in dimethyl sulfoxide (DMSO) solution and diluted to aCSF. Indomethacin was dissolved with 4% sodium bicarbonate solution. L-NAME was dissolved in saline.

Surgery

Adult male Sprague-Dawley rats, weighing 250~300 g, were used and were housed individually at an ambient temperature of 22±1°C with a 12 h light-dark cycle. Animal water and food *ad libitum* were allowed. After anesthesia with secobarbital sodium (30 mg/kg, i.p), a cannula (0.8 mm o.d.) was stereotaxically implanted in the lateral ventricle (P: 0.9 mm, L: 1.5 mm, V: 3.5 mm) for intracerebroventricular (i.c.v.) injection of drugs, according to Paxinos and Watson (Paxinos et al, 1997). The cannula was anchored with dental cement to the calvarium surface. The reflected muscles and skin were replaced around the mound containing the cannula and were sutured. These animals were used for experiment after the recovery period for 3~5 days.

Determination of effects on body temperature

Experiments were conducted between 10 : 00 a.m. and 7 : 00 p.m.. The rectal temperature of each rats was measured at every 30 min in concious state. Only animals whose body temperature was stable and in the range of 36.5~37.5°C were used to determine the effect of applied drug.

Thermal indexes were calculated as areas under the curves (°C/h) after the treatments for a total period of 6 hrs.

Statistics

Temperature responses were assessed as changes from pre-injection values (⊿°C). Results were expressed as the means±S.E.M. for experiments. The significance of the difference between groups was determined by Student's *t*-test.

RESULTS

The basal body temperatures of the experimental groups did not differ significantly from those of the control group.

I.c.v. injection of NO donor SNP (0.04~4 nmol) produced dose-dependent febrile response in rats that started to increase 1 hr after the injections, whereas vehicle (aCSF) caused no significant change in body temperature. Thermal indexes (TI) showed that SNP at the dose of 0.4 nmol (TI=1.25±0.18°C/hr) and 4 nmol (TI=1.41±0.22°C/hr) caused a significant increase in body temperature compared with the group injected with vehicle (TI=0.31±0.14°C/hr) (Fig. 1).

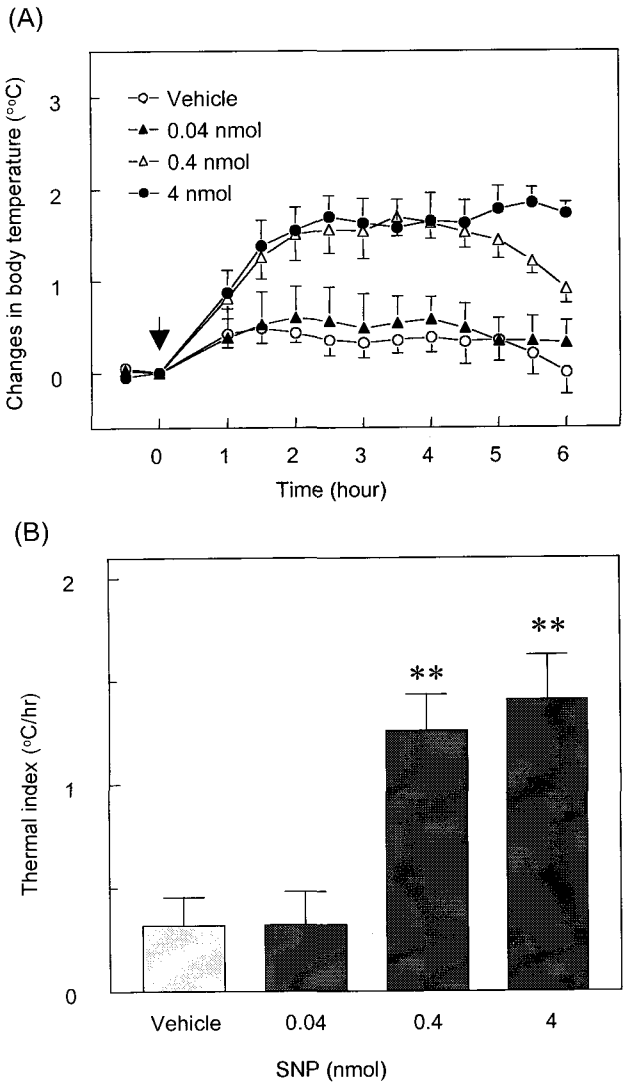

Fig. 1. Effect of i.c.v. administered SNP on the body temperature. (A) Changes in body temperature. (B) Thermal index for 6 hours after injection of SNP shown in (A). Each value represents the mean±S.E.M. of 4~7 experiments. Arrow indicates time of injection. Vehicle: 3 μl of artificial cerebrospinal fluid. **P<0.01 compared with vehicle group.

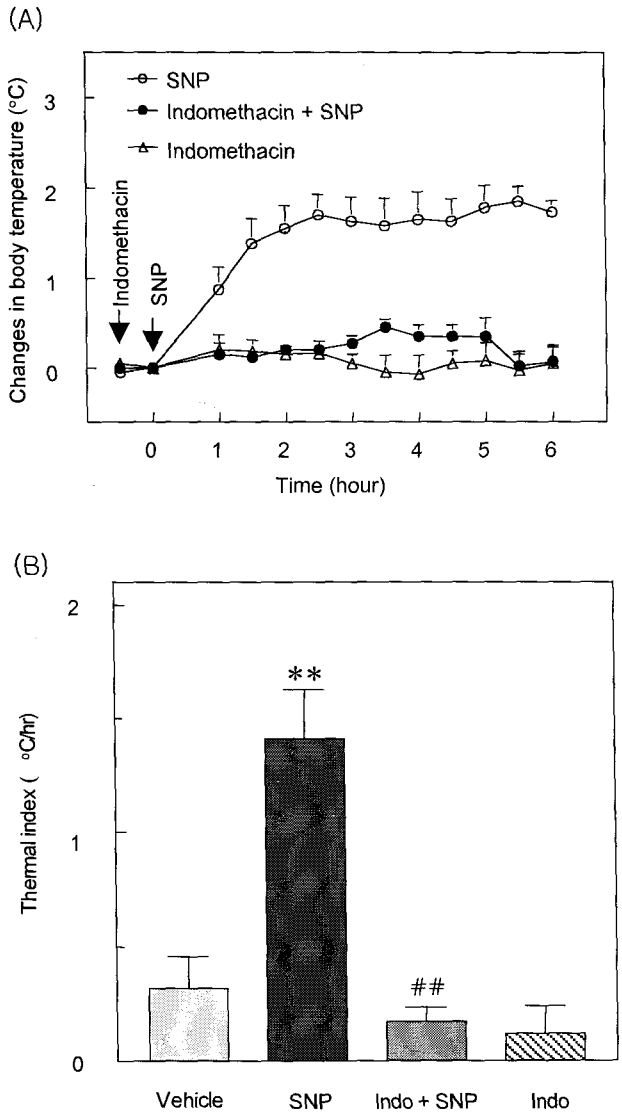

Fig. 2. Effects of indomethacinon the SNP-induced febrile response. (A) Changes in body temperature. (B) Thermal index for 6 hours after injections of SNP shown in (A). Indomethacin (10 mg/kg, i.p.) was injected 30 min before injection of SNP (4 nmol, i.c.v). Each value represents the mean ± S.E.M. of 4 ~ 5 experiments. Arrow indicates time of injection. **P < 0.01 compared with vehicle group. ##P < 0.01 compared with SNP alone group.

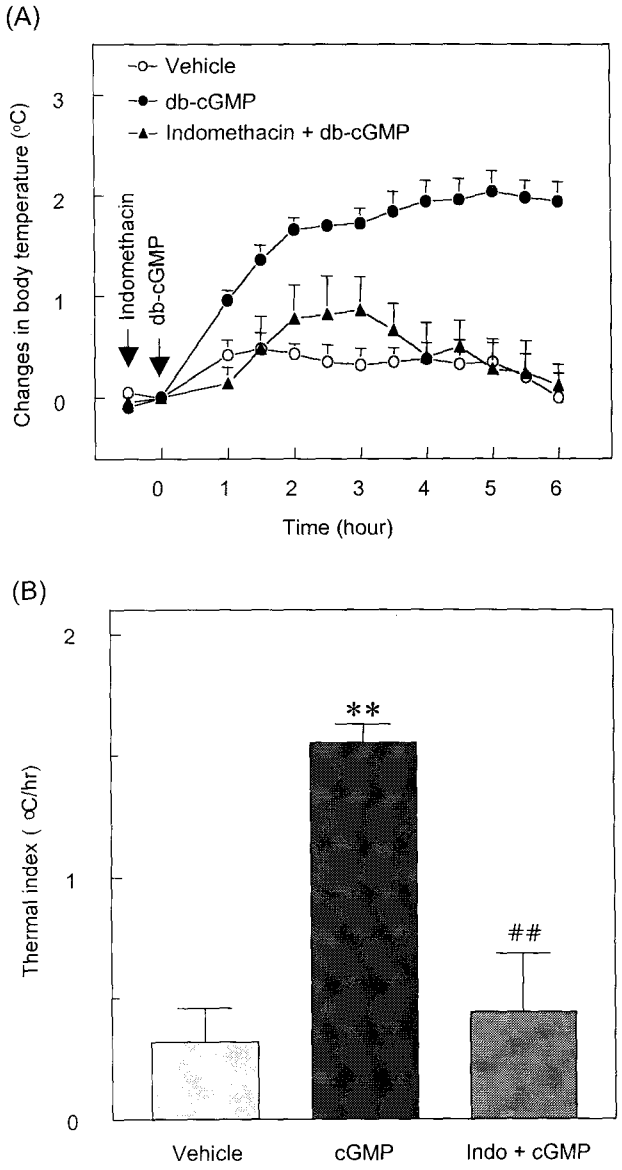

Fig. 3. Effect of cGMP and indomethacin on the body temperature. (A) Changes in body temperature. (B) Thermal index for 6 hours after injections of cGMP shown in (A). Indomethacin (10 mg/kg, i.p.) was injected 30 min before injection of cGMP. Each value represents the mean ± S.E.M. of 4 ~ 5 experiments. Arrow indicates time of injection. Vehicle: 3 μl of artificial cerebrospinal fluid. db-cGMP: dibutyryl cGMP 100 μg (i.c.v). **P < 0.01 compared with vehicle group. ##P < 0.01 compared with cGMP alone group.

Pretreatment of rats with indomethacin (10 mg/kg, p.o, n=4) significantly attenuated the fever induced by i.c.v. injection of SNP (4 nmol) in rats (TI=0.17 ± 0.07°C/hr) (Fig. 2).

The membrane permeable analogue of cyclic GMP, dibutyryl cGMP (100 μg, i.c.v.) produced significant febrile response in rats (TI=1.55 ± 0.08°C/hr) and this effect was completely attenuated by pretreatment with indomethacin (TI=0.44 ± 0.24°C/hr) (Fig. 3). However, SNP induced fever was not reduced by i.c.v. pretreatment with ODQ 50 μg (TI=1.33 ± 0.18°C/hr) (Fig. 4).

The body temperature was markedly increased by i.c.v injection of IL-1 β 10 ng (TI=1.75 ± 0.27°C/hr).

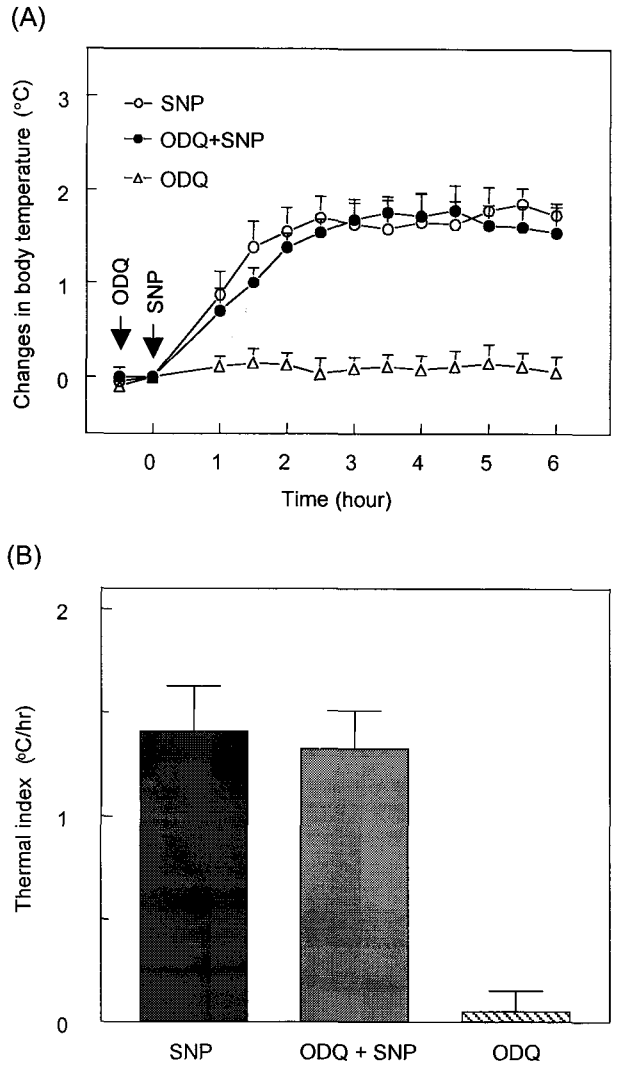

Fig. 4. Effects of guanylyl cyclase inhibitor on the fever induced by SNP. (A) Changes in body temperature. (B) Thermal index for 6 hours after injections of SNP shown in (A). ODQ (50 μg, i.c.v.) was injected 30 min before injection of SNP (4 nmol, i.c.v.). Each value represents the mean±S.E.M. of 4~5 experiments. Arrow indicates time of injection.

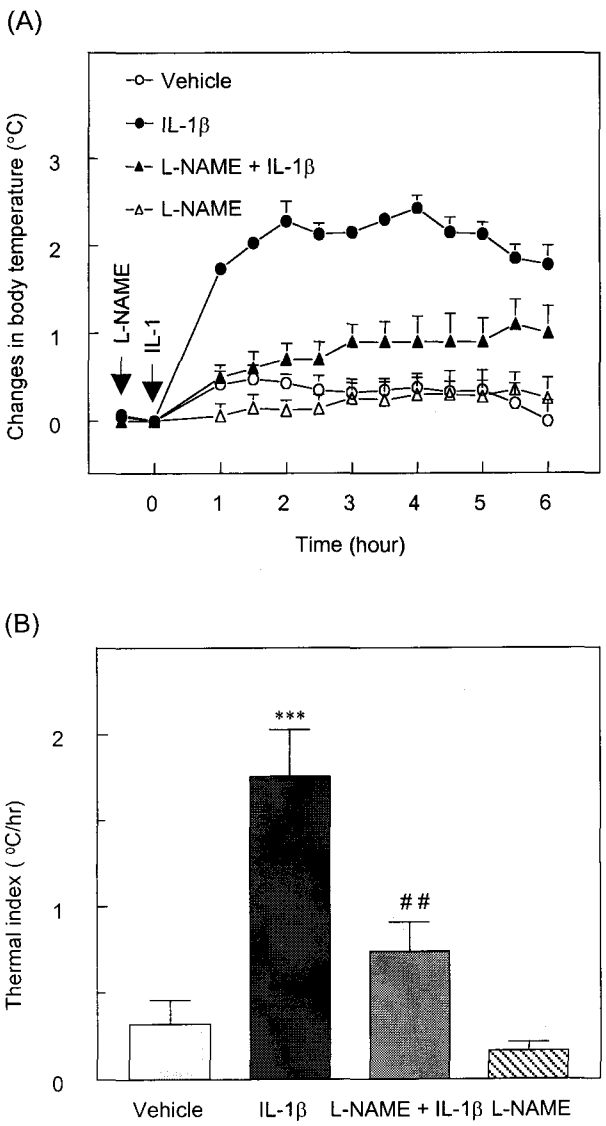

Fig. 5. Effects of L-NAME on the IL-1 β-induced febrile response. (A) Changes in body temperature. (B) Thermal index for 6 hours after injections of IL-1 β shown in (A). L-NAME (50 mg, i.p.) was injected 30 min before injection of IL-1 β (10 ng, i.c.v.). Each value represents the mean±S.E.M. of 4~5 experiments. Arrow indicates time of injection. ***P<0.01 compared with vehicle group. ##P<0.01 compared with IL-1 β alone group.

The IL-1 β-induced fever was significantly attenuated by pretreatment of L-NAME (50 mg/kg, i.p.). NO synthase inhibitor alone had no effect on body temperature (TI=0.16±0.05°C/hr) (Fig. 5).

DISCUSSION

There are several studies about the roles of NO on febrile response and thermoregulation. The intravenously injected NOS inhibitor L-NAME produced a stereoselective, dose-dependent hypothermia and reduced the febrile response to the intravenously injected lipopolysaccharide (Scammell et al, 1996). Also, the intraperitoneally injected IL-1 β-induced febrile response was completely suppressed by simultaneous i.p. injection of L-NAME (Roth et al, 1998). In contrast, Kapas et al (1994) reported that the pyrogenic

action of either intracerebroventricularly or intravenously injected IL-1 was not affected by L-NAME, and Raghavendra et al (1999) reported that the intracerebroventricular coadministration of L-NAME enhanced LPS-induced hyperthermia. Thus, we studied the effect of centrally administered NO donor on thermoregulation in conscious rats to elucidate the role of centrally produced NO in febrile response.

The i.c.v. injection of NO donor SNP caused a dose-related increase in body temperature.

The ending step in febrile response is the action of PGE_2 on thermoregulatory pathways in the hypothalamus (Coceani, 1990; Dinarello, 1990), and increased PGE_2 may move the set-point to higher level. The inhibition of SNP-induced pyresis by indomethacin means that effect of SNP was exerted through the set-point mechanism in hypothalamus.

Many effectors of NO production lead to the simultaneous release of mediators (such as prostaglandin E_2) from the cyclooxygenase pathway. This is true for the agents such as LPS or IL-1 β (Salvemini et al, 1990; Stabler et al, 1991). NO stimulates cyclooxygenase in various in vitro models, including short-term incubations of rat medio-basal hypothalami (Rettori et al, 1992; Salvemini et al, 1993). More recently, however, it has been demonstrated that NO can also inhibit cyclooxygenase activity (in particular, the inducible isoform COX-2) in microglial cells (Minghetti et al, 1996). Moreover, the possibility that NO activates cyclooxygenase in the same manner as it does soluble guanylyl cyclase (i.e. binding to the heme moiety of the enzyme) has been questioned (Tsai, 1994). Though the issue of the actions exerted by NO on cyclooxygenase remains under dispute, in any case, cyclooxygenase inhibition blocked the fever induced by SNP.

Several effects of NO are mediated by cGMP through the stimulation of soluble guanylate cyclase. The present results show that pretreatment with ODQ, an inhibitor of guanylate cyalase (Schramnel et al, 1996), does not attenuate the fever induced by i.c.v injection of IL-1 β or SNP. Soluble guanylate cyclase is virtually absent from the rat hypothalamus (Matsuoka, 1992; Burgunder et al, 1994). The effects of NO on prostaglandins production have to date been described as cyclic GMP-independent (Salvemini et al, 1993). This suggests that the fever induced by NO may be cyclic GMP-independent pathway. But, i.c.v injection of dibutyryl cyclic GMP, a membrane permeable analogue of cyclic GMP, causes a febrile response. The increase of cyclic GMP may activate phospholipase A_2 to provide arachidonic acid, the substrate for conversion by the activated cyclooxygenase to prostaglandin E_2 (Canteros et al, 1995). These results raise the prospect that cyclic GMP formed within the tissue of the brain has a supplementary role in a febrile response.

We also investigated NOS inhibitor on the i.c.v. injected IL-1 β-induced febrile response. Similar to SNP-induced pyresis, IL-1 β-induced hyperthermia also significantly attenuated by L-NAME.

These results propose that NO may have an important role in pathway of thermoregulation in hypothalamus and NO-induced fever is independent on soluble guanylate cyclase.

REFERENCES

Banks WA, Ortiz L, Plotkin SR, Kastin AJ. Human interleukin (IL) 1 α, murine IL-1 α and murine IL-1 β are transported from blood to brain in the mouse by a shared saturable mechanism. *J Pharmacol Exp Ther* 259: 988−996, 1991

Burgunder JM, Cheung PT. Expression of soluble guanylyl cyclase gene in adult rat brain. *Eur J Neurosci* 6: 211−217, 1994

Canteros G, Rettori V, Franchi A, Cebral E, Faleti A, Gimeno M, McCann SM. Ethanol inhibits luteinizing hormone-releasing hormone (LHRH) secretion by blocking the response of LHRH neuronal terminals to nitiic oxide. *Proc Natl Acad Sci USA* 92: 3416−3420, 1995

Coceani F. Prostaglandins and fever: facts and controversies. In: Mackowiak P, ed, *Fever: basic mechanisms and management.* Raven Press, New York, p 39−70, 1990

Dinarello CA. Endoenous pyrogens. The role of cytokines in the pathogenesis of fever. In: Mackowiak P, ed, *Fever: basic mechanisms and management.* Raven Press, New York, p 23−47, 1990

Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and relased from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 84: 9265−9269, 1987

Kapas L, Shibata M, Kimura M, Krueger JM. Inhibition of nitric oxdie synthesis supresses sleep in rabbits. *Am J Physiol* 266: R151−R157, 1994

Luheshi GN, Gay J, Rothwell NJ. Circulating IL-6 is transported into the brain via a saturable transport mechanism in the rat. *Br J Pharmacol* 112: 636P, 1994.

Matsuoka I, Giuili G, Poyard M, Stengel D, Parma J, Guellaen G, Hanoune J. Localization of adenylyl and guanylyl cyclase in rat brain by in situ hybridization:

comparison with calmodulin mRNA distribution. *J Neurosci* 12: 3350−3360, 1992

Minghetti L, Polazzi E, Nicolini A, Creminon C, Levi G. Interferon-gamma and nitric oxide down-regulate lipopolysaccharide-induced prostanoid production in cultured rat microglial cells by inhibiting cyclooxygenase-2 expression. *J Neurochem* 66: 1963−1970, 1996

Nagashima T, Ohinata H, Kuroshima A. Involvement of nitric oxide in noradrenaline-induced increase in blood flow through brown adipose tissue. *Life Sci* 54: 17−25, 1994

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 3rd edit. Academic Press. Sydney. 1997

Raghavendra V, Agrewala JN, Kulkarni SK. Role of centrally administered melatonin and inhibitors of COX and NOS in LPS-induced hyperthermia and adipsia. *Prostagl Leukot Essent Fatty Acids* 60: 249−253, 1999

Rettori V, Gimeno M, Lyson K, McCann SM. Nitric oxide mediates norepinephrine-induced prostaglandin E_2 release from the hypothalamus. *Proc Natl Acad Sci USA* 89: 11543−11546, 1992

Rivier C, Shen G. In the rat, endogenous nitric oxide modulates the response of the hypothalamic-pituitary-adrenal axis to interleukin-1 β, vasopressin, and oxytocin. *J Neurosci* 14: 1985−1993, 1994

Roth J, Storr B, Voigt K, Zeisberger E. Inhibition of nitric oxide synthase results in a suppression of interleukin-1 β-induced fever in rats. *Life Sci* 62: PL345−PL350, 1998

Salvemini D, Korbut R, Anggard E, Vane J. Immediate release of a nitric oxide-like factor from bovine aortic endothelial cells by Escherichaia coli lipopolysaccharide. *Proc Natl Acad Sci USA* 87: 2593−2597, 1990

Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. Nitric oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci USA* 90: 7240−7244, 1993

Scammell TE, Elmquist JK, Saper CB. Inhibition of nitric oxide synthase produces hypothermia and depresses lipopolysaccharide fever. *Am J Physiol* 271: R333−338, 1996

Schrammel A, Behrends S, Schmidt K, Koesling D, Mayer B. Characterization of 1H-[1,2,4]oxadiazolo [4, 3-a]quinoxalin-1-one as a heme-site inhibitor of nitric oxide-sensitive guanylyl cyclase. *Mol Pharmacol* 50: 1−5, 1996

Stabler J, Racic MS, Billiar TR, Curran RD, Mcintyre LA, Georgescu HI, Simmons RL, Evans CH. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. *J Immunol* 147: 3915−3920, 1991

Stitt JT. Prostaglandin E as the neural mediator of the febrile response. *Yale J Biol Med* 59: 137−149, 1986

Tsai A. How does NO activate hemeproteins? *FEBS Lett* 341: 141−145, 1994