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Nitric oxide as a peripheral and central mediator in temperature regulation

E. Simon

Max-Planck-Institute for Physiological and Clinical Research, Bad Nauheim, Federal Republic of Germany

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Summary. In animals including humans nitric oxide (NO) serves as a biological messenger both peripherally at neuroeffector junctions and in the central nervous system where it modulates neuronal activity. Evidence for the involvement of NO in homeostatic control is accumulating also for temperature regulation in homeotherms. In the periphery an auxiliary role in the vasomotor control of convective heat transfer to heat dissipating surfaces and modulation of thermoregulatory heat generation, especially in brown adipose tissue as the site of nonshivering thermogenesis, are discussed as NO actions. At the central level a thermolytic role of NO in thermoregulation as well as in fever is assumed, however, experimental data opposing this view suggest that topical specificity may be important. At the level of single neurons, the observed interrelationships between thermosensitivity and responsiveness to NO are still not consistent enough to reconcile these data with the effects of NO-donors and inhibitors of NO-synthase on temperature regulation.

Keywords: Nitric oxide – Temperature regulation – Fever – Body temperature – Thermoregulatory effectors – Central neurons – Nitric oxide synthase – Cyclic GMP

Introduction

Temperature regulation in homeotherms is accomplished by a distributed system of neurons with the hypothalamus as the highest level of integration (Hammel, 1968). Temperature signals are provided by a multiple-input system comprising skin thermoreceptors and thermosensors in deep-body tissues, especially in the central nervous system (CNS) where they extend from the preoptic and anterior hypothalamic (POAH) region throughout the brainstem and the spinal cord (Simon et al., 1986). Somatomotor and autonomic efferents control diverse thermoregulatory effectors which became involved in the evolution of homeothermy. The multiplicity of nervous components controlling thermoregulatory activities implies participation of a multitude of transmitters or modulators. This should include nitric oxide

(NO) as a more or less ubiquitous signal molecule, and data concerning its role in temperature regulation are beginning to emerge.

The presence of NO-Synthase (NOS) in the autonomic nervous system has been amply documented by demonstrating its NADPH-diaphorase activity or by immunocytochemistry (Rand and Li, 1995). At the central level, the widespread distribution of neurons and fiber systems containing NOS or NO-activated guanylate cyclase producing the second messenger cyclic GMP (cGMP) have been demonstrated for the hypothalamus (Bredt et al., 1990; DeVente and Steinbusch, 1992). At the spinal level both are present around the central canal and in the superficial laminae of the dorsal horn where neurons are involved in the transmission and possibly generation of temperature signals (Valtschanoff et al., 1992; Schmid et al., 1997).

Pharmacology of NO in temperature regulation and fever

Vascular smooth muscle relaxation is a key action of NO in the peripheral circulation. In the non-adrenergic, non-cholinergic, thermoregulatory control of blood flow in the rabbit ear skin and in the heat dissipating nasal mucosa of dogs NO seems to exert a permissive function (Farrell and Bishop, 1995, Watanabe et al., 1995), but not in humans (Dietz et al., 1994). NO may also be relevant in the process of cold acclimation as a local messenger in the brown adipose tissue (BAT), a heat generating thermoregulatory effector organ (Kuroshima, 1995).

At the central level, intracerebroventricular (icv.) application of an NOS-inhibitor stimulated the sympathetic innervation of BAT indicating that NO might be an inhibitor of cold defense (De Luca et al., 1995). This action should imply a corresponding activation of heat defense consisting, in panting animals, in increased skin blood flow and an associated rise in skin temperature, increased respiratory rate and an associated rise in respiratory evaporative water loss, no change or a reduction in metabolic heat production and, as a result, in a decrease of deep body temperature. This was, indeed, demonstrated in rabbits infused icv. with a NO-donor (Fig. 1A). The closely similar effect of intravenous (iv.) infusion of NO-donors in rabbits (Fig. 1B) may be explained by its peripheral vasodilatatory action and possible additional central effects, since NO readily permeates the blood-brain barrier. For fever, as a pathophysiological up-regulation of body temperature, analogous studies indicate a similar antipyretic action of NO, although the available evidence is not fully conclusive. As summarized in Fig. 2, fever induced by lipopolysaccharide (LPS) in rabbits was enhanced by icv. application of a NOS-inhibitor and attenuated by icv. application of a NO-donor (Gourine, 1995). When rabbits were pretreated with iv. infusions of the drugs, LPS-induced fever was enhanced by NOS-inhibitor infusion but did not change significantly during NO-donor infusion. In rats, the rise in body temperature caused by icv. injection of E-type prostaglandin (PGE1), a presumed central mediator of fever, was attenuated by co-application of the NO-precursor L-arginine or of a NO-donor (Monda et al., 1995). In cats NOS-blockade did not effectively alter the

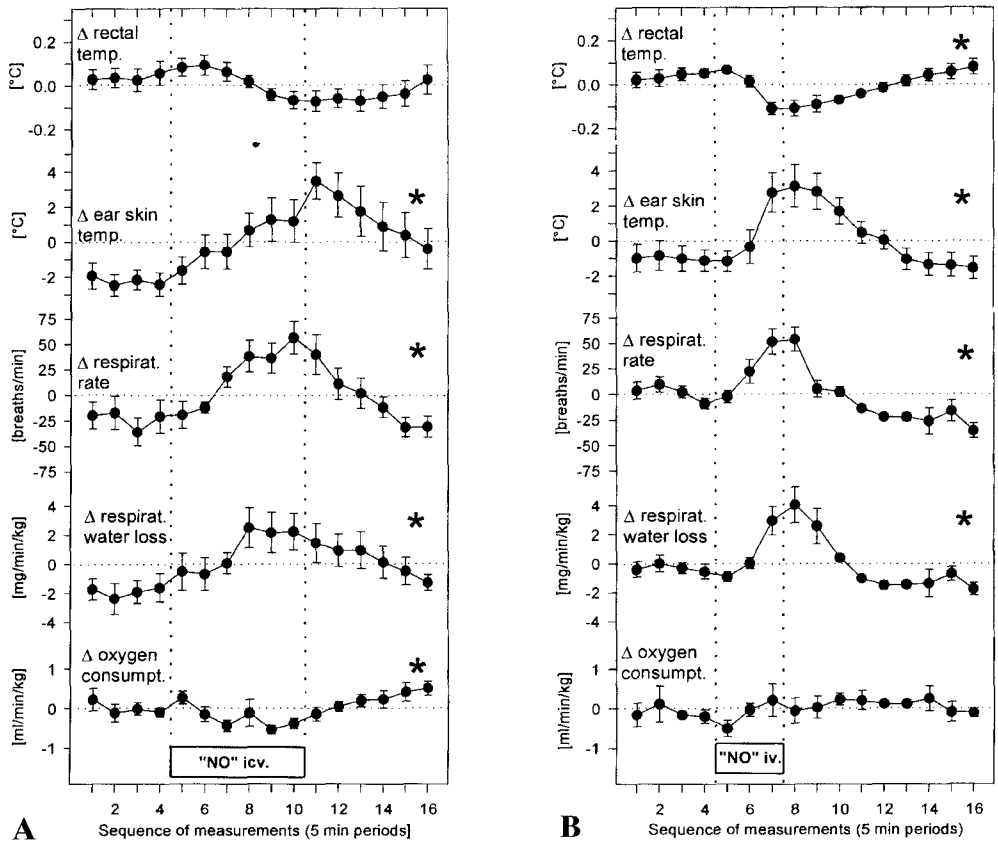

Fig. 1. Effects of NO-donors infused icv. (**A**) for 30 min (5–10 nmol/min/kg of 3-morpholino-sydnonimine-hydrochloride, 7 experiments) or iv. (**B**) for 15 min (75 nmol/min/kg of 3-morpholino-sydnonimine-hydrochloride or S-nitroso-N-acetylpenicillinamine, 6 experiments) in conscious rabbits exposed to a slight external heat load (ambient temperature 24°C). Shown are the deviations with standard errors from the average levels of the thermoregulatory parameters determined during 16 successive measuring periods of 5 min duration starting 20 min before the start of NO-donor infusions. Asterisks: significant treatment effect (ANOVA). Data from Eriksson et al., 1997 and Mathai et al., 1997

fever response (Redford et al., 1995).Taken together, the currently available evidence tends to be in favor of a thermolytic function of NO as a messenger involved in the central coordination of thermoregulatory effectors. However, the effector responses associated with the hypothermic and antifebrile actions to iv. infusions of a NO-donor in rabbits were found to be not fully coordinated (Gagalo et al., 1995), raising the suspicion that reactions secondary to the peripheral hypotensive action of NO may interfere with its central thermolytic action. This might also explain the observation in rats that fever induced by systemically applied LPS was converted into a hypothermic response by a co-applied NOS blocker (Scammell et al., 1996).

In detail, stimulation of cold defense by NO when acting at specific sites may oppose its general thermolytic action as suggested by the following

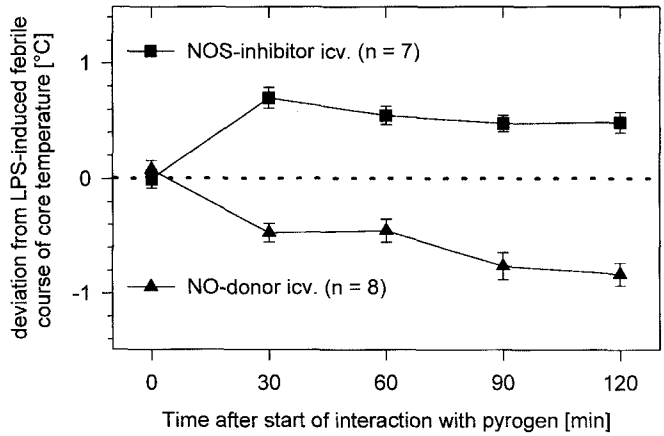

Fig. 2. Effects of icv. injections of NOS-inhibitors or NO-donors on fever induced in conscious rabbits by iv. injection of LPS. Means with standard errors. Shown are the deviations from the LPS-induced rise of core (rectal) temperature during 120 min after the start of interaction between LPS and drugs, initiated either by LPS injection iv. after the animals had been injected icv. with the NOS-inhibitor or by icv. injection of the NO-donor 60 min after iv. injection of LPS. Upward deviation from zero line: enhanced fever; downward deviation: reduced fever (Data from Gourine, 1995)

observations. Injection of a NOS-inhibitor directly into the thermosensory POAH region of anesthetized rats reduced activation of BAT by PGE2 (Amir et al., 1991). In rabbits the involvement of inducible NOS was concluded from dose-dependent attenuation of LPS fever by certain NOS-inhibitors and from hyperthermic actions of NO-donors when locally injected into the organum vasculosum laminae terminalis (OVLT) which is considered as the most important interface in the transmission of the pyrogenic signal from the circulation to the CNS (Lin and Lin, 1996). Thus, NO actions on central receptive structures may differ directionally from its effects in the central thermointegrative neuronal network.

Thermosensitivity and NO-responsiveness of central neurons

Presently detailed data concerning NO-responsiveness of thermosensitive neurons in CNS regions known to function as thermosensory sites are available only for the spinal cord (Table 1). Among warm sensitive neurons in the superficial dorsal horn (laminae I + II) which are located in the thermoafferent pathway, combined phasic/static responses prevailed, similar to peripheral thermoreceptors, whereas purely static responses prevailed among warm sensitive neurons in lamina X close to the central canal. NO actions were also different in that a majority of lamina I + II neurons were inhibited but most of the lamina X neurons were activated. At the hypothalamic level, a preliminary *in vivo* study on anesthetized rats receiving icv. microinjections of NO-donors or a NO-blocker reported partly excitation and partly inhibition of neurons

Table 1. A Average temperature coefficients of warm sensitive neurons and fraction with combined phasic/static temperature responses in laminae I + II and in lamina X recorded *in vitro* from spinal cord tissue slices (data from Pehl et al., 1997). **B** Distribution of inhibitory and excitatory actions of the NO-donor sodium nitroprusside among neurons in laminae I + II and in lamina X recorded *in vitro* from rat spinal cord tissue slices (data from Pehl and Schmid, 1997)

A. Temperature response	Temperature coefficient (mean ± standard error) [imp/s/°C]	Combined phasic/static response [%]
Laminae I + II (n = 79)	1.6 ± 0.1	73[a]
Lamina X (n = 55)	1.8 ± 0.1	9[a]

B. Effect of NO-donor	Inhibition [%]	Stimulation [%]	No effect [%]
Laminae I + II (n = 90)	49[a]	28[a]	23
Lamina X (n = 84)	2[a]	93[a]	5

[a] Significantly different fractions (chi-square test).

recorded in the thermosensory POAH region (Gourine et al., 1995), and among these neurons inhibition by interleukin-1 in 2 cases was opposed by the NO-donor which, vice versa, opposed excitation by interleukin-1 in one case, i.e., effects were observed which would comply with the antipyretic action of NO. A greater sample of neurons recorded *in vitro* from hypothalamic tissue slices demonstrated exclusively inhibition of warm sensitive as well as insensitive POAH neurons by application of a NO-donor, while in a nearby portion of the diagonal band of Broca neurons were insensitive to the NO-donor and thereby indicated a site-specific expression of NO-responsiveness in hypothalamic structures (Schmid et al., 1997). Inhibition of warm sensitive neurons in thermosensory regions would be in line with evidence for an activation of cold defense by NO but at variance with its thermolytic effects in thermointegrative regions.

Perspective: functional morphology of NO in temperature regulation

The neurophysiological elucidation of the thermoregulatory network within the CNS faces the fundamental difficulty that knowledge about its cytoarchitecture reaching beyond the coarse topography of the brain regions involved, is virtually non-existing. In this situation NO, like any mediator acting within this network in a seemingly specific manner, provides a highly welcomed opportunity to advance cytoarchitectural knowledge by localizing its sites of generation as well as of its actions at the cellular level. Attempts to find short-term effects of LPS on NOS-activity in the OVLT have failed, so far (Sehic et

al., 1997). However, most recently enhanced NOS activity was shown to accompany the site-specific augmentation the cFOS gene product in the peripheral zone of the OVLT and the diagonal band of Broca of rats subjected to heat acclimation at 33°C for 2 days (Gerstberger, 1998). Thus, this approach appears worthwhile being pursued, including the identification of putative cellular targets of NO and its second messenger(s) by analyzing calcium transients in viable cells of organotypic cultures or cGMP formation by immunocytochemistry.

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Author's address: Prof. E. Simon, M.D., Max-Planck-Institute for Physiological and Clinical Research, D-61231 Bad Nauheim, Federal Republic of Germany.

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