Numerous studies made during the past decade have identified nitric oxide (NO) as a widespread messenger molecule in the control of immunological and autonomic body functions. Formerly known as the endothelium-derived relaxing factor, NO was first shown to be generated by the isoform of the enzyme, nitric oxide synthase (NOS), that is constitutively expressed in vascular endothelial cells (eNOS) including those of brain vessels, whereas evidence for eNOS expression in neurons is inconsistent and, so far, limited to a subset of hippocampal cells. The second constitutive NOS isoform exists in central and peripheral nervous cellular elements (nNOS), and its endothelial expression is restricted to pial blood vessels. An inducible isoform (iNOS) becomes expressed in macrophages and presumably in other nucleated cell types, including (non-neuronal) cells in the central nervous system (CNS), in response to cytokines and exogenous pyrogens (5). The modes of action of NO, being a gaseous and diffusible molecule enzymatically derived from L-arginine as its natural precursor, deviate from those of conventional messenger molecules such as amino acids, amines, or peptides. First, NO is neither stored in nor released from presynaptic secretory vesicles; rather, it is generated on demand. Second, its range of action corresponds to its range of diffusion, which may encompass many target cells, and is not confined to the standard prepostsynaptic direction of signal transmission (15). Third, NO mainly acts by diffusing into cells, where it changes enzymatic activities. Its main physiological target is soluble guanylate cyclase, and cGMP is the second messenger mediating most of its actions. Under other, especially pathological, circumstances NO interactions with other enzymes and superoxide anions may become important.

The ubiquity of the various isoforms of NOS suggests that NO is involved in the control of many cellular activities. However, disruption of either the eNOS or nNOS gene in mice was found to be compatible with apparently normal pre-and postnatal development (8), suggesting mutual compensation as long as the diffusible messenger NO is generated, especially in the CNS. Alternatively, functions of NO may be taken over by other messengers in unknown ways; this reflects the redundancy in the control of many biological functions. Although this might exclude that a certain form of NOS is essential for the life of an animal in the laboratory (a highly protective, artificial living condition), it by no means excludes important roles for NO as an essential transmitter or just modulator in optimizing body performance under conditions of internal or external challenge. Especially in the CNS, NO is involved in the mediation of endocrine and autonomic nervous activities and contributes to long-term adjustments of synaptic function (15). Thus it is safe to assume that the NOS-NO-cGMP signal transduction system among neurons contributes, to a greater or lesser degree, to any major homeostatic activity, including the control of body temperature. The latter is the topic of this review. It is an area that has only recently begun to be studied extensively but holds much promise of yielding meaningful new information on thermoregulatory mechanisms.

**Distribution of NOS in the CNS as an outline for thermoregulatory analysis**

Perturbations of heat balance are among the most common challenges with which homeotherms, including humans, have to cope. They can be caused either by unfavorable climatic conditions or by altered generation of internal heat. Moreover, most host-defense responses to bacterial and viral challenges involve fever (1). The multiplicity of inputs, controllers, and effectors in the thermoregulatory system is well docu-
mented. Nervous control of body temperature is accomplished by distributed neuronal networks, with the hypothalamus as the thermoregulatory center, and auxiliary integrative functions at lower brain stem and spinal cord levels, i.e., in parts of the CNS in which only nNOS is expressed in neurons and in which the NO target guanylate cyclase is abundant (12,15). Among the multiple thermosensory inputs providing the integrated feedback signal for body temperature control, those originating in the skin and in deep-body thermosensors outside the CNS are relayed in the superficial dorsal horn with its dense network of NOS-containing ("nitroxidergic" or "nitrergic") fibers and cell somata, where, in addition, spinal temperature signals are assumed to be generated. This review focuses on NO as a central nervous messenger in temperature regulation. The relevant data were mostly obtained by pharmacological approaches: compounds releasing NO (NO donors) or antagonizing its synthesis (NOS inhibitors) were shown to change body temperature per se or, in addition, altered thermoregulatory effector activities in ways that explained the temperature changes. Because thermoregulatory adjustments in response to physiological stimuli occur with latencies on the order of seconds to several minutes, only constitutively expressed NOS should presumably be involved. The still fragmentary and, in part, controversial observations currently preclude a straightforward definition of the role of NO in temperature regulation. However, they might justify a first working hypothesis, especially if the also still fragmentary, but accumulating, histochemical and neurophysiological data about the topographical differences in the control of nNOS and about site-specific NO actions in the CNS are taken into consideration.

### NO-related pharmacological studies of thermoregulation and fever

In different animal species, various NO donors and NOS inhibitors were applied by different routes to study the responses of single thermoregulatory effectors and of core temperature, or their coordinated changes, under conditions of normothermia, heat stress, or fever. Table 1 classifies the currently available data according to whether they indicate that NO lowers or increases body temperature and

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>Site of Application</th>
<th>Mode of Action</th>
<th>Reference</th>
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<tr>
<td>Rat</td>
<td>ip</td>
<td>↑</td>
<td>De Luca et al., 1996</td>
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<tr>
<td>Rat</td>
<td>iv</td>
<td>↑</td>
<td>Scammell et al., 1996</td>
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<td>Rabbit</td>
<td>iv</td>
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<td>Farrell and Bishop, 1995</td>
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<tr>
<td>Rabbit</td>
<td>iv</td>
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<td>Mathai et al., 1997</td>
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<tr>
<td>Horse</td>
<td>iv</td>
<td>↓</td>
<td>Mills et al., 1997</td>
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<tr>
<td>Rat</td>
<td>icv</td>
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<td>De Luca et al., 1996</td>
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<td>Eriksson et al., 1997</td>
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<tr>
<td>Rat</td>
<td>POAH</td>
<td>(-)</td>
<td>Amir et al., 1991</td>
</tr>
<tr>
<td>Rabbit</td>
<td>OVLT</td>
<td>↑</td>
<td>Lin and Lin, 1996</td>
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**Table 1. Effects of NO donors and NOS inhibitors on deep body temperature as shown by change in activities of thermoregulatory effectors in normothermic or febrile animals**

Sites of application: iv, intravenous; ip, intraperitoneal; icv, intracerebroventricular (3rd or lateral ventricle); OVLT, organum vasculosum laminae terminalis; POAH, preoptic and anterior hypothalamic region. Pyrogenic agents: LPS, lipopolysaccharide; IL-1, interleukin-1; PGE, prostaglandin E. ↑, Presumed or actually observed rise; ↓, decrease; (-), no clear effect. References are from Ref. 12.
reduces or enhances fever. As discussed elsewhere in more detail (12), it would appear from the responses to systemic drug applications that NO may have a role as a peripheral mediator controlling single thermoregulatory effectors. However, when administered peripherally, the NO donor enhanced skin blood flow, whereas the NOS inhibitor reduced sympathetic control of brown adipose tissue thermogenesis and thereby suggested that locally acting NO may change thermal balance in opposite directions depending on the effector involved. Responses to drugs applied into the CNS suggest a role for NO as a modulator of neuronal thermoregulatory activity. Furthermore, NO liberated from systemically applied NO donors might well reach central nervous targets because of its high diffusibility. For NOS inhibitors, it is explicitly or implicitly assumed in many studies that they also penetrate the blood-brain barrier (BBB)—at least some of them or at higher doses and with some delay. It is apparent from the data in Table 1 that thermoregulatory responses to pharmacologically induced changes in central NO availability are inconsistent during both normothermia and fever.

In a few studies, all major autonomic thermoregulatory effectors and body temperature were monitored simultaneously in experiments in which NO availability was changed experimentally. The data summarized in Fig. 1 (from Refs. 2 and 7) indicate coordinated heat-loss responses induced by NO not only when NO donors are administered into the third cerebral ventricle (icv) but also when they are given intravenously (iv). Because the thermoregulatory responses to central and peripheral drug application were so similar, the most likely explanation would be that they were both mediated centrally. However, accompanying cardiovasculo-

![Graphical representation](http://physiologyonline.physiology.org/Downloadedfrom)
lar responses were probably caused by NO acting on different targets, because a decrease in blood pressure and tachycardia were induced with the iv route and an increase in blood pressure and bradycardia with the icv route of NO donor application. Not shown are data obtained with iv NOS inhibitors, which produced responses opposite to those elicited by the NO donors (7).

Site-specific NO actions in the hypothalamic thermoregulatory network are suggested by the course of core temperature in normothermic or febrile rabbits (6) in which NO donors or NOS inhibitors were injected into the organum vasculosum laminae terminalis (OVLT), a preoptic brain site outside the BBB considered to be an important receptive structure in the mediation of pyrogen- or cytokine-induced fever (1). Microapplication of NO donors into normothermic rabbit OVLT caused hyperthermia by increasing cutaneous vasoconstriction and/or increasing thermogenesis; this effect was attenuated by local pretreatment with methylene blue as an inhibitor of guanylate cyclase and hemoglobin as an NO scavenger. However, when in the same study fever was stimulated by microinjection of lipopolysaccharide (LPS) into the rabbit OVLT, several NOS inhibitors preinjected 1 h before LPS had inconsistent effects. Some of the NOS inhibitors suppressed the fever response, but others did not; suppression of inducible rather than constitutive NOS seemed to be involved in antipyresis. On the other hand, analogous studies on rabbits with direct injection of the drugs into the third cerebral ventricle with NO donors or an NOS inhibitor indicate excitation of some and inhibition of other neurons in the preoptic anterior hypothalamus (POAH) region where thermanesory structures are presumed to be most densely concentrated (4). In this region inhibition of two neurons and excitation of another neuron by interleukin-1, an established fever-generating cytokine, were opposed in each case by NO-donor administration, i.e., effects were induced that were consistent with an antipyretic action of NO. In a larger sample of neurons recorded in vitro from hypothalamic tissue slices, 17 POAH neurons tested were exclusively inhibited by slice superfusion with the NO donor sodium nitroprusside, irrespective of whether they were warmth sensitive, but in the nearby ventromedial part of the preoptic area, none of 4 neurons was inhibited by this NO donor. Thus, similar to the spinal cord, site-specific differences in NO responsiveness seem to exist in hypothalamic structures as well (12).

The reviewed data obtained by the pharmacological approach strongly suggest that NO is effective as a central modulator of temperature regulation. This view is supported by observations that changes in central NO availability change the activities of thermoregulatory effectors in a coordinated manner so that they act in concert to decrease or increase body temperature. On the other hand, the inconsistencies found with respect to the direction of the induced body temperature changes preclude any definitive conclusion about the role of NO as a hypothermic or antipyretic rather than a hyperthermic or pyretic agent; rather, the data suggest that the site specificity of NO actions in hypothalamic structures may account in part for the observed diversity, although species differences must be taken into consideration. This view seems to be supported by the few currently available electrophysiological data about site-specific NO actions on thermanesensitive and -insensitive central neurons and by new histochemical observations that suggest a role of central nNOS in heat-stress responses.

**Thermosensitivity and NO responsiveness of central neurons**

Detailed data concerning NO responsiveness of thermanesensitive neurons in CNS regions known to function as thermanesory sites are available only for neurons in the superficial laminae I and II of the dorsal horn and in the region around the central canal (lamina X) of the spinal cord. Warmth-sensitive neurons in lamina X exhibit exclusively static temperature responses (proportional relationship between temperature and discharge rate), whereas a majority of those in laminae I and II display combined phasic/static responses (enhanced discharge rate during the phase of rising temperature) (10, 11). This differently expressed warmth sensitivity combines with a lamina-specific distribution of NO responsiveness (9), because the large majority of lamina X neurons are activated by NO donors, whereas the majority of neurons in laminae I and II are inhibited (Fig. 2). Both actions involve cGMP as the intracellular messenger.

At the hypothalamic level, preliminary data from anesthetized rats injected in the third cerebral ventricle with NO donors or an NOS inhibitor indicate excitation of some and inhibition of other neurons in the preoptic anterior hypothalamus (POAH) region where thermanesory structures are presumed to be most densely concentrated (4). In this region inhibition of two neurons and excitation of another neuron by interleukin-1, an established fever-generating cytokine, were opposed in each case by NO-donor administration, i.e., effects were induced that were consistent with an antipyretic action of NO. In a larger sample of neurons recorded in vitro from hypothalamic tissue slices, 17 POAH neurons tested were exclusively inhibited by slice superfusion with the NO donor sodium nitroprusside, irrespective of whether they were warmth sensitive, but in the nearby ventromedial part of the preoptic area, none of 4 neurons was inhibited by this NO donor. Thus, similar to the spinal cord, site-specific differences in NO responsiveness seem to exist in hypothalamic structures as well (12).

Taken together, the pharmacological analysis of thermoregulatory activities and the available electrophysiological data suggest, as a first working hypothesis, that NO might cause hyperthermia and enhance fever by influencing neuronal activity oppositely at sites where pyrogens act, as

"...NO is effective as a central modulator of temperature...."
in the OVLT, or where temperature is monitored, as in the POAH and the superficial laminae of the dorsal horn. Activation of heat loss and antipyresis were clearly expressed when NO donors were applied into the third cerebral ventricle, where they acted presumably at sites of thermointegration. Thus the electrophysiological data for site-specific NO effects in the thermoregulatory network of the CNS seem to be in line with the working hypothesis derived from the pharmacological analysis of temperature regulation. However, support by independent structural information is urgently needed. Fortunately, morphological data have been obtained very recently that, indeed, open new perspectives for future functional studies.

**Perspective: Functional morphology of the NO signal cascade in temperature regulation**

Attempts to elucidate the thermoregulatory functions of nitrergic neuronal systems within the CNS have heretofore faced the fundamental difficulty that knowledge about the cytoarchitecture of the central thermoregulatory network, beyond the broad topography of the brain regions involved, was virtually nonexistent. However, NO, which acts within this network in a seemingly specific manner, can now be traced histochemically in neurons by marking either the enzyme by which NO is generated (NOS) or the product (cGMP) of the enzyme, soluble guanylate cyclase, that is the main neuronal target of NO. This provides a new opportunity to analyze the thermoregulatory neuronal network by localizing sites of NO generation as well as sites of its actions at the cellular level under conditions of thermoregulatory challenge.

Stimulation of iNOS expression in central nervous glial elements by pyrogens and cytokines points to a role of NO in host defense, but the latency of iNOS induction seems to preclude a major role of this central NO source in the early phases of (experimental) fever and in physiological temperature regulation. Attempts to find short-term effects of pyrogens on neuronal nNOS activity in the OVLT, as a fever-mediating target, have failed so far (13). However, preliminary observations suggest that nNOS may be influenced by longer lasting thermal loads. Thus, in the rostral brain stem of rats, enhanced enzymatic NADPH-diaphorase staining, which exclusively represents the nNOS isoenzyme as proven by parallel immunocytochemistry confirming cellular nNOS and excluding eNOS and iNOS expression, can be detected in rats after heat exposure (34°C ambient temperature; 2 days) in discrete preoptic sites. Most distinctly enhanced is nNOS activity in the rostral brain stem of rats, anterior hypothalamic structures involved in thermoreception and -integration.

**FIGURE 2.** Examples of different effects of NO on discharge rate of thermosensitive neurons recorded extracellularly in lamina X (A) or lamina II (B) from a spinal cord tissue slice. *Middle traces*, discharge rate; *bottom traces*, slice temperature. Superfusion of slice with NO donor sodium nitroprusside (SNP), at a concentration of 10^{-5} M (black bar), excited (A) or inhibited (B) the respective neurons. *Top graphs*, relationships between tissue temperature and steady discharge. Temperature coefficient (TC; impulses s^{-1} °C^{-1}) was calculated by linear regression from experimental periods indicated by circled numbers. During NO-mediated inhibition of the lamina II neuron, warmth sensitivity was reduced. Graphical presentation of data from Ref. 11 (with permission).

“...NO...can now be traced histochemically in neurons...”
like the MPA and the VMPO showed five- and ninefold increases, respectively, in the number of Fos protein-immunopositive cell nuclei after two days of heat exposure, indicative of thermally induced neuronal activation (Fig. 3, right). In numerous neurons in the VMPO, the c-fos gene product and cytosolic nNOS are colocalized in the very same cell. Hypothalamic structures known to be involved in central osmoreception such as the subfornical organ or the OVLT only revealed cell nuclei immunopositive for the Fos protein after water deprivation for 24 h, causing mild extracellular dehydration. Neurons in the median preoptic nucleus, an important relay station in body fluid homeostatic control, were activated equally by heat acclimation and mild dehydration; the two effects were additive. This differential responsiveness strongly suggests functional specificity of both Fos and nNOS activation.

At the spinal level, the observed distribution of NOS activity and NO-induced cGMP activity strongly suggests, in combination with the electrophysiological data, that NO is involved in the transmission and generation of peripheral and local spinal temperature signals. A parallel analysis of thermally induced changes in neuronal activity with electrophysiological techniques and

FIGURE 3 A: schematic drawing of a midsagittal section showing thermo- and osmoreponsive structures in anteroventral hypothalamus and lamina terminalis of the rat brain. AC, anterior commissure; MI, massa intermedia; MnPO, median preoptic nucleus; MPA, medial preoptic area; OC, optic chiasm; OVLT, organum vasculosum laminae terminalis; SFO, subfornical organ. B: NADPH-diaphorase staining in a coronal brain section of a heat-exposed rat (2 days; 34°C) showing constitutively expressed neuronal nitric oxide synthase (nNOS)-positive neurons in OVLT and ventromedial preoptic area (VMPO). C: left, quantification of NADPH-diaphorase staining in OVLT, VMPO, and islands of Calleja (IC), a structure not involved in thermo- or osmoregulation (n = 4) and animals exposed to 34°C for 2 days (n = 4), using computerized optical density measurements of diaphorase staining product in the respective hypothalamic regions (RAG-200, BioCom-system) after appropriate calibrations. Right, number of Fos-immunopositive cell nuclei in OVLT and VMPO of control rats and heat-exposed animals. In each group, 4 animals were studied and counts were obtained from 8–10 histological sections for each structure. Fos immunoreactivity remained unaltered in hypothalamic structures unrelated to the central thermoregulatory circuit such as the subfornical organ or supraoptic nuclei (Patronas et al., Brain Res. 798: 127–139, 1998). For statistics, one-way ANOVA was complemented by post hoc multiple comparisons (Newman-Keuls), with level of significance set at *P < 0.05.
by means of NOS and cGMP histochemistry might help to identify the neurons that contribute specifically to the thermosensory input.

**Conclusion**

Evidence for the involvement of NO in the homeostatic control of body temperature in homeotherms is accumulating. In the periphery, NO may play an auxiliary role in the vasomotor control of convective heat transfer by the blood to heat-dissipating surfaces and may modulate thermoregulatory heat generation by brown adipose tissue as the site of nonshivering thermogenesis. At the central level, the currently available evidence suggests topical specificity of NO actions in physiological temperature regulation as well as in fever. The available experimental data support the view that NO decreases body temperature when it acts at sites involved in thermointegration but increases body temperature when it acts at sites of pyrogen- and temperature perception within the CNS. NO may excite as well as inhibit central neurons. At least for the spinal cord, a distinct topography could be demonstrated for these opposite NO actions. In addition to the pharmacological analysis of thermoregulatory effector responses in vivo and to studies of neuronal responsiveness in vivo and in vitro performed with the help of NO donors and NOS inhibitors, the histochemical evaluation of nNOS in conditions of thermal challenge has recently been started. The combination of these methods appears to be a promising approach to unravel the still largely unknown cytoarchitecture of the neuronal networks by which temperature regulation is accomplished.

*The invaluable help of Prof. E. Simon in the preparation of this manuscript is highly appreciated.*

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"...NO actions in physiological temperature regulation..."