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Gaseous neurotransmitters and their role in anapyrexia

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Abstract

Mammals keep their body temperature (Tb) relatively constant despite important changes in their metabolic rate. However, in some particular situations it may be beneficial to increase or to decrease Tb, in a relatively more significantly way. For instance, under hypoxic conditions, a regulated drop in Tb (anapyrexia) takes place which has been reported to be crucial for survival in a number of different species. This review highlights major advances in the research about nitric oxide and carbon monoxide (where data are relatively less abundant), before focusing on the role played by the gaseous neuromediators in thermoregulation, under the conditions of euthermia and anapyrexia. Available data are consistent with the notion that both NO and CO, acting in the CNS (intracerebroventricular approach), do participate in thermoregulation, NO decreasing Tb and CO increasing it. However further studies are required before definitive conclusions can be made, as to their physiological mechanisms of action.

Keywords

Hypothermia; Anapyrexia; Ectothermy; Endothermy; Neural pathways; Neuromediators; Preoptic area

1. Introduction

The efforts towards the identification and characterization of a potent vasodilating substance produced by endothelial cells in the 1980s ended up providing a new paradigm in research and human health and disease. It has been a hallmark the motion that the endothelial cell-derived relaxing factor (EDRF) was a soluble gaseous molecule (Furchgott and Zawadzki 373-76) and not a peptide, protein, lipid mediator, or nucleic acid as one would normally expect. The impact of NO in biomedical research and applications to human diseases since the discovery of NO has been astounding.

It is interesting to note that it was in 1968, i.e., about 20 years before NO was suggested as EDRF, when Tenhunen et al (Tenhunen, Marver, and Schmid 748-&) reported that cells can produce another endogenous gaseous molecule by an endogenous enzymatic reaction (the catalytic breakdown of heme by the microsomal heme oxygenase (HO) enzyme producing carbon monoxide (CO), iron and bilirubin. This finding remained relatively quiescent, unnoticed by the scientific community, for about 25 years. Needless to say, we are disregarding the fact that CO administration in high amounts induces the formation of blood carboxyhemoglobin, causing tissue hypoxia and thus can be lethal.

Over the years, evidence has been growing in support of the importance of NO and CO as neuromodulators involved in the control of body temperature.

2. Nitric oxide

The discovery of endothelium-derived relaxing factor by Furchgott and Zawadzki, further identified as the gas nitric oxide (NO), gave origin to the concept that a gaseous compound may be a signaling molecule in biological systems (Furchgott and Zawadzki 373-76). It is currently known that endogenously formed NO arises from the catabolism of L-arginine, resulting in the formation of L-citrulline and NO, a reaction catalyzed by the enzyme NO synthase (NOS) (Garthwaite 2783-802). Active NOS enzymes are dimeric and their activity also requires other factors such as NADPH, tetrahydrobiopterin (BH4), FAD, FMN, heme and calmodulin (CaM). Three major isoforms of nitric oxide synthase are known: nNOS or NOS1, iNOS or NOS II and eNOS or NOSIII. nNOS and eNOS are constitutively expressed enzymes and their activity is Ca^{2+}-dependent. Expression of iNOS is induced in response to inflammatory stimuli (LPS, for instance) and the enzyme is not regulated by Ca^{2+}. Actually it has calmodulin associated to it. After synthesized, NO takes part in a number of physiological processes such as smooth muscle relaxation, blood pressure and volume regulation, platelet aggregation, immunomodulation, axon outgrowth and guidance mechanisms as well as cellular growth, apoptosis, proliferation, differentiation, and neurotransmission. NO is produced on demand and is not stored as other messengers. However, NO complexes may exist as stored precursors to release NO (Works et al. 3728-39).

Like other free radicals, NO is highly reactive. In vivo concentrations of NO can range from low nanomolar to low micromolar. NO targets and cellular actions depend on its local concentrations and the availability of the target molecules. Nonetheless, one can state that at low nanomolar concentrations, the activation of soluble guanylyl cyclase (sGC), the major NO receptor, results in the elevation of intracellular cyclic GMP (cGMP) (Steiner and Branco s1398-s1408). This process is thought to be the major event triggered by low concentrations of NO. Other low NO concentration targets are transcription factors, Cytochrome C oxidase, and Catalase as well as thiol groups in various proteins which are nitrosated by NO on the Cystein residues (Erusalimsky and Moncada 2524-31). NO synthases may also produce superoxide anion ($O_2^{\bullet -}$) or reactive nitrogen species other than NO (Alderton, Cooper, and Knowles 593-615). At higher concentrations, NO rapidly reacts with superoxide anion to form the very reactive peroxinitrite causing nitration of proteins involved in diverse cellular physiological processes(Gonzalez, Drapier, and Bouton 43345-51;Bian et al. 5712-17).

Therefore, the actions of NO can be classified into two different ways: cGMP-dependent and cGMP-independent. Here we review data providing solid evidence that when it comes to thermoregulation (focus will be given in the maintenance of euthermia and anapyrexia) the effects of NO seem to be cGMP-dependent. This process involves the activation of sGC.

sGC is a heterodimer with an alpha and beta subunits with a ferrous heme liganded to histidine 105 of the beta subunit. In the absence of NO, soluble GC exhibits very low basal

activity. Conformational changes following the binding of NO to heme result in marked activation of the enzyme. When NO binds to the ferrous iron, it changes the heme liganding to the enzyme, and activates the enzyme from 200- to 400-fold while decreasing the Michaelis Menton Constant (Km) for guanosine triphosphate as substrate (Murad 2003-11). The resulting elevation in the intracellular cGMP concentrations triggers the activation of a number of signal transduction pathways that are responsible for regulating a number of physiological processes. cGMP can produce its effects by activating protein kinases that phosphorilate serine or threonine residues of a variety of proteins. Three isoforms of cGMP-dependent protein kinase (PKG) have been described, *i.e.*, 2 soluble isoforms and a particulated isoform. Protein phosphorilation can modify the structure and function of proteins or their enzymatic activity *cf.* (Csiszar 465-79).

One may wonder how does an elevation of cellular cyclic GMP levels resulting from the activation of sGC by NO regulate in so many different physiological processes. One possible explanation may reside on the fact there are a number of different cell types and their specific spatiotemporal regulation of sGC activity and the choice of signaling cascades regulated by NO-cGMP, may also be involved. Thus, it sounds fairly suitable to discuss the NOS activity regulation and localization.

Actually, NOS regulation has been one of the most extensively studied subjects (Madhusoodanan and Murad 681-94). The expression of eNOS was first observed in endothelial cells but later it has been reported to be also present in other cell types (Pollock et al. 10480-84). Neuronal nitric oxide synthase (nNOS), as the name suggests is primarily found in the brain, albeit it is also expressed in other tissues such as the skeletal muscle (Perotti et al. 1381-87). Moreover, it has been reported that NO may play a role by increasing contractile function (Kobzik et al. 546-48), and glucose transport in skeletal muscle (Balon and Nadler 359-63). Once it is well established that skeletal muscle activity is closely related to body temperature, Peroti et al suggested that nNOS inhibition could induce hypothermia by decreasing the heat production from skeletal muscle.

As mentioned before both the NOS isoform eNOS and nNOS are Ca^{2+}-dependent enzymes and eNOS respond to CaM binding with an increased rate of electron transfer from NADPH to flavins bound at the reductase domain (Ilagan et al.). Consequently eNOS and nNOS isoforms respond to local Ca^{2+} oscillations. On the other hand (in the absence of infection), expression of iNOS is undetectable in most tissues. In response to inflammatory signals, iNOS is induced in many cell types including neuronal and glial cells, and its activity is not sensitive to changes in Ca^{2+} levels.

There are examples for receptor stimulated activation of NOSs. Factors such as glutamate, histamine and acetylcholine, have also been reported to stimulate the production of NO under a physiological conditions. Moreover, in endothelial cells, kinins can induce the synthesis of NO by stimulating B1 and B2 receptors (Campos and Calixto 902-09) whereas steroid hormones such as estrogen can also stimulate NO synthesis from eNOS by activating G-protein $G_{\alpha}i$ (Wyckoff et al. 27071-76).

Evidence are that NOS activities may also be regulated through protein–protein interactions and lipid modifications. Such protein–protein interactions and posttranslational modifications are thought to allow NOS to act on specific intracellular compartments, permitting a spatial resolution of signals transmitted by NO to take place (Ortiz and Garvin 107-14;Liu, Hughes, and Sessa 1525-35). As recently reported, in the central nervous system neurons, nNOS is recruited to the site of the NMDA receptor activation through its protein–protein interactions (Miguel and Nunes-de-Souza 39-46), and NMDA receptors is known to be involved in thermoregulation (Ding et al. 201-08).

Localization of NO production within the CNS, similarly to its regulation, may have important implications. The tissue distribution of NOS may mediate multiple effects. For instance, NOS inhibition in the anteroventral preoptic region of the rat brain results in an increased febrile response indicating a antipyretic role of the NO-cGMP pathway in the AVPO (Steiner et al. R584-R593), whereas the NOS pathway in organum vasculosum laminae terminalis has been reported to play a pyrogenic role (Lin and Lin 347-52). As to the systemic effect of NO, Kozak and Kozak used a genetical models to assess the differential roles of nitric oxide synthase isoforms in fever using NOS gene-deficient mice (Kozak and Kozak 2534-44) and they found that nitric oxide was indeed a regulator of fever, but its action would differ depending on the pyrogen used and the NOS isoform. More recently, we used iNOS-KO mice to study the role of No in the tolerance to LPS, and we found that NO modulates LPS tolerance in mice and that iNOS isoform is involved in NO synthesis during LPS tolerance (Dias et al. 1322-27). Recently data obtained from mice lacking all the three NOSs have been reported (Morishita et al. 10616-21). Data regarding body temperature control using this animal are eagerly awaited with interest.

NO-cGMP signaling may be also regulated more down stream in the NOS pathway, *i.e.*, at the level of sGC. Studies on the tissue distribution of sGC have shown that its expression levels may vary among different tissues (Sulakhe et al. 705-12), and that the expression level of different α and β subunits may also vary among the examined tissue. For instance, distribution of α_2 subunit has its major occurrence in brain (Gibb and Garthwaite 539-44), which is consistent with reports on the developmental appearance of cyclic GMP production and NO responsiveness (Currie, de Vente, and Moody 1668-77). Unfortunately, the physiological significance of the differences in the expression levels and distribution of these subunits is still unknown.

Expression of sGC may also be regulated posttranscriptionaly. At least in smooth muscle cells, a cyclic AMP-dependent (Gerassimou et al. 1084-91) has been reported. Moreover, there are evidence to support the notion the regulation of mRNA for sGC α and β subunits is dependent on both cyclic AMP and cyclic GMP (Kloss, Srivastava, and Mulsch 1440-51). Consistently with the putative role of cyclic AMP and cyclic GMP, the degree of phosphorylation, dephosphorylation and the protein–protein interactions also have an effect on the activity of sGC (Ilagan et al.). It is important to mention that, once again, the possible physiological implication of such processes remains unknown, including regarding thermoregulation.

A recent study reported that localized activation of NOS and sGC can also be achieved by means of protein–protein interactions. The interaction of the synaptic protein PSD-95 with sGC is dependent of the recruitment of sGC to the synaptic membranes, where nNOS is located (Russwurm, Wittau, and Koesling 44647-52). At the synaptic membrane proteins forms a signaling complex with the cytoplasmic domain of the NMDA receptor. Although the recruitment of sGC to the site of nitric oxide synthesis by PSD-95 is well accepted, the contribution of such a phenomenon to the overall activation of sGC and elevation in cellular cGMP levels observed after NMDA receptor activation is not completely understood *cf.* (Jurado, Sanchez-Prieto, and Torres 3165-75).

Regulation of cyclic GMP production may also be regulated by time. Such temporal regulation is achieved by means of a tightly regulated activation and desensitization cycles. When NO is present, sGC is instantaneously activated in milliseconds with an half maximal effective concentration (EC50) for NO of about 45 nM. Dissociation of NO from sGC may mark the inactivation of the enzyme (Bellamy and Garthwaite 165-76).

In summary, the signal diversity and specificity observed for NO synthesis and regulation of sGC activity includes multiple factors, which provides room for a scenario where a high degree of spatial and temporal resolution exists. Such signals downstream of cGMP can be transmitted by downstream effectors. Cyclic GMP regulates these processes by means of three direct effectors: 1) protein kinase G (PKG), 2) cyclic nucleotide phosphodiesterases (PDEs) and 3) cyclic nucleotide gated ion channels. By their turn, each of these effectors can transmit their signals to a number of intracellular signaling molecules (regulating neurotransmission, for instance). It is important to mention that all of these effectors of cGMP have been found to be expressed in brain (as well as other tissues) and their role in regulating nervous system functions is in general relatively well studied. Unfortunately, once again, the knowledge of their physiological role (including thermophysiology) remains poorly understood. Following is a small discussion given for each one of the three effectors.

PKG was one of the first proteins to be identified as a target of cGMP (cf. Murad Can J Ophthalmol. 2008, in press). It is very well established that activation of PKG by cGMP is a major mechanism by which NO relaxes smooth muscle tissue. Latter, following the discovery of the activation of sGC by the NO donor sodium nitropruside, it has been established that cGMP synthesized in nervous system works as a powerful neurotransmitter (Weight, Petzold, and GREENGAR.P 942-44). Then PKG has been reported to be widely expressed in many parts of the brain. To date, two isoforms of PKG have been reported, PKGI (PKG-Iα and PKG-Iβ) and PKG-II (Uhler 13586-91) (Elhusseini, Bladen, and Vincent 2814-17). Both PKG-I and PKG-II contains catalytic and regulatory domains. Among these, PKG-I is primarily cytosolic, where as PKG-II is myrystoylated and is generally found in membrane associated form. As to the putative isoform related to inflammatory stimuli PKG-I has recently been reported to play a role in mice (Tegeder et al. 3253-57).

Cytosolic cGMP can change neuronal excitability by activating cyclic nucleotide gated ion channels channels, which may play a crucial role in the signal transduction pathway involved in the modulation of various functions by NO (Hofmann, Biel, and Kaupp 587-89).

Activation of these channels causes cell depolarization and then helps excitation of neurons. Moreover, there are reports suggesting that they may permit a significant Ca2+ influx that may influence synaptic function (Parent et al. 3295-301). Although the expression of CNG channels have also been reported in several parts of the brain including hippocampus the exact mechanisms of their regulation or their physiological function is not known.

Cyclic nucleotide signalling is modulated not only by cAMP and cGMP, but also by the rate of cyclic nucleotide degradation via phosphodiesterases (PDEs) (Beavo, Conti, and Heaslip 399-405). Phosphodiesterase superfamily include a number of subfamilies (about 11) with around twice as much genes that are transcribed into more than 50 enzyme species. Practically phosphodiesterases have been reported to be expressed in the CNS (Menniti, Faraci, and Schmidt 660-70). Cyclic GMP can be degraded not only by several cGMP-specific phosphodiesterases but also by dual-substrate phosphodiesterases that hydrolyse both cAMP and cGMP. The scenario looks far from simple, once cGMP may also inhibit or activate specific PDE subtypes by binding to their regulatory domains. Thus, the nucleotide may actually affect its own intracellular concentration. Finally, an unique characteristic has been reported: there seem to be a cross-talk between the Ca2+ and cyclic nucleotide signalling pathways once they can be activated by the binding of Ca2+/calmodulin (Ogiwara, Chik, and Ho 95-102).

NO may also signal through a cGMP-independent way. For instance it may give rise to free radical species which can affect cellular functions by means of a number of mechanisms (Stamler et al. 691-96). NO can also affect proteins responsible for neuronal functions by stimulating their ADP ribosylation (Sullivan, Wong, and Schuman 414-24) which may account at least in part for the stimulatory effect of NO on neurotransmitter release in some synapses (Schuman and Madison 153-83;Garthwaite 2783-802). Moreover, NO may also affect cellular functions by S-nitrosylation of various proteins that can be associated with their cAMP response (Riccio et al. 283-94), or with modulations of ion channels function(Jian et al. 481-85).

Besides the CNS, the NOS enzymes are widely distributed throughout the body (Steiner and Branco 263-88). Since NOS is encountered in various tissues involved in body temperature (T_b) regulation, it seems plausible that this gas influences thermoregulation. Actually, we and others have provided evidence that NO plays differential thermoregulatory effects by acting at the periphery and in the CNS. This notion is based on the opposite results obtained by injecting pharmacological modifiers of the NO pathway systemically or intracerebroventricularly (icv)(Bicego, Barros, and Branco 616-39).

2.1. NO in the periphery

Studies on rats in their thermoneutral zone have shown that the systemic nonselective inhibition of NO synthesis using L-arginine analogues at doses ranging from 10 to 30 mg/kg decreases T_b (Bicego, Barros, and Branco 616-39;Steiner and Branco 263-88;Steiner and Branco s1398-s1408), despite the fact that NOS inhibitors should decrease cutaneous heat loss because it causes vasoconstriction of both large and small vessels, including the superficial vascular beds. Thus, it has been suggested that NO synthesis inhibition is likely to reduce T_b by causing a failure of thermogenic mechanisms. Actually, inhibition of NO

synthesis has been shown to impair brown adipose tissue thermogenesis (Cannon and Nedergaard 277-359). It seems that nNOS is at least one NOS isoform involved in thermogenesis since intraperitoneal (ip) administration of the nNOS inhibitor 7-nitroindazole (7-NI) at the dose of 30 mg/kg evokes a drop in T_b of rats similar to that obtained with nonselective NOS inhibitors (Cannon and Nedergaard 277-359;Perotti et al. 1381-87).

In contrast to the data obtained with rats, rabbits treated systemically with the nonselective NOS inhibitor N^G-nitro-L-arginine-methyl ester (L-NAME) presented a rise instead of a drop in T_b, which was accompanied by decreased respiratory heat dissipation (Mathai et al. R1691-R1697). Taken as a whole, these results support the notion that the thermoregulatory effect produced by systemic inhibition of the NO pathway depends on the prominent thermoregulatory effector mechanism in the tested species. More studies exposing animals to cold and warm environments and, thus, selecting the most manifest effector mechanism, are necessary to firmly establish this notion.

Recently, the physiological roles of constitutively expressed NOS isoforms in humans, in vivo, have been assessed. 7-NI seems to attenuate cutaneous vascular conductance increases in response to whole-body heat stress, but not during local skin warming. These opposite effects of 7-NI on two NO-dependent processes may suggest that the nNOS isoform affects NO increases and hence vasodilatation during centrally mediated, reflex responses to whole-body heat stress, but not during locally mediated, axon reflex responses to local skin warming (Kellogg, Zhao, and Wu 847-57).

The involvement of peripheral NO in febrigenic signaling to the brain has been proposed because peripherally administered NOS inhibitors attenuate LPS-induced fever. However, it seems that NO is uninvolved in febrigenic signaling to the brain (Steiner et al. 1204-13).

2.2. NO in the CNS

NO acting on the brain has important thermoregulatory effects. A number of studies have observed that icv injection of L-NAME at doses of about 250 μg causes a slight increase in the T_b of rats, indicating that central NO plays a tonic role by reducing T_b (Simon 87-93).

This hypothermic role of NO in the central sites is likely to be mediated by activation of soluble guanylate cyclase and consequent rise in the intracellular levels of cyclic GMP since icv administration of the soluble guanylate cyclase inhibitor ODQ (1 μg) elevates T_b similarly to NOS inhibitors. One could argue that ODQ could also affect T_b by inhibiting carbon monoxide-dependent soluble guanylate cyclase activity. However, this is unlikely since we observed that central inhibition of the carbon monoxide pathway causes no change in T_b (Steiner et al. R584-R593).

It is interesting to note that central NO has been shown to play a role in reducing sympathetic tonus by acting in several brain sites, including the paraventricular nucleus, posterior hypothalamus and the nucleus tractus solitarious (Monda et al. 489-93). Since sympathetic fibers play a key role in both increasing nonshivering thermogenesis and in evoking vasoconstriction of the superficial vascular beds, responses which lead to an

increase in T_b, it is suitable to propose that a reduction in the sympathetic outflow by centrally acting NO may be responsible for the hypothermic action of NO in the CNS.

The effect of NO on body temperature seems to be mediated by the activation of the serotonin-cAMP and NO-cGMP pathways in the preoptic region. The first studies (Steiner and Branco s1398-s1408) suggesting a participation of cyclic nucleotides in the regulation of T_c were reported in the 1970s. More specifically, these studies suggested that administration of cAMP analogs that mimic cAMP-like effects into the preoptic region (PO), which is the presumed brain T_c controlling site (Steiner et al. R584-R593), increased T_c. However, these observations started to be contested in 1984, when it was reported that intra-PO administration of cAMP and cGMP analogs to rabbits produces a rapid decrease in T_c followed by a feverlike response (Steiner and Branco s1398-s1408). Interestingly, the fever, but not the decrease in T_c, was abolished by treatment with paracetamol, indicating that cAMP and cGMP reduce T_c by acting on the PO and that the pyretic effect of intra-PO cAMP observed in previous studies is likely to result from a local inflammatory response produced by the injection procedure (Steiner and Branco s1398-s1408).

More recently studies using small volume microinjections have confirmed that intra-PO administration of cAMP and cGMP analogs that activate protein kinases A and G, respectively, produces a decrease in the T_c of rats (Steiner, Rocha, and Branco R1412-R1422;Steiner, Antunes-Rodrigues, and Branco 0135-45). Consistent with this notion, cAMP increases the thermosensitivity of warm-sensitive preoptic neurons, an effect that seems to be associated with increased heat loss mechanisms and a decrease in T_c (Wright et al. R1704-R1715). Moreover, the use of small volume microinjections also permitted the identification of the anteroventral PO (AVPO) as the preoptic site most sensitive to the thermoregulatory effects of cyclic nucleotides (Steiner et al. R584-R593;Steiner, Antunes-Rodrigues, and Branco 135-45). Actually, we have shown that the activation of cAMP- and cGMP-dependent pathways in the PO mediates hypoxia-induced anapyrexia. Inasmuch as the rise in cAMP during anoxia seems to be under the control of the monoaminergic system (Zamboni et al. 164-68), whereas rises in cGMP may be driven by nitric oxide (NO; for a review, see Ref. (Garthwaite 2783-802)). It is interesting to note that recently Wright et al reported data supporting these results obtained by means of intra-nucleus microinjection, using immunohistochemistry to showed that rostral hypothalamic neurons contain cGMP, guanylate cyclase, and CNG A2 (an important cyclic nucleotide-gated channel). They also measured extracellular electrophysiological activity from different types of neurons in rat hypothalamic tissue slices in response to 8-bromo-cGMP (a membrane-permeable cGMP analog). The cGMP analog decreased the spontaneous firing rate in 45% of temperature-sensitive and -insensitive neurons, an effect that is likely due to cGMP-enhanced hyperpolarizing K(+) currents (Wright et al. R1704-R1715).

Recently Felender et al reported that NE released in the POA in response to the vagally conveyed pyrogenic message of PGE_2 generated by complement component 5a-stimulated Kupffer cells mediates the characteristic biphasic T_c rises evoked by LPS in conscious guinea pigs (Feleder, Perlik, and Blatteis R1135-R1143). These data adds to the role of NO in the POA reported earlier (Steiner et al. R584-R593).

3. Heme-oxygenase

HO catabolizes heme into carbon monoxide (CO), biliverdin (which is rapidly converted to bilirubin), and free iron (which leads to the induction of ferritin, an iron-sequestering protein). To date, three HO isoforms (HO-1, HO-2, and HO-3) have been documented to catalyze this (Ryter, Alam, and Choi 583-650). HO-1 is a 32-kDa protein that is inducible by numerous stimuli including heme but also nonheme stimuli such as heavy metals, hormones, LPS, cytokines (at leats interleukin [IL] 10) and oxidants (hydrogen peroxide) (Ryter, Alam, and Choi 583-650). This diversity of HO-1 inducers has provided further support for the speculation that HO-1, besides its role in heme degradation, may play a vital function in maintaining cellular homeostasis (Bilban et al. 267-79).

Data obtained from experiments using *hmox-1* $^{-/-}$ null “knockout” mice suggest that HO-1 may be a key molecule in the host’s defense, once the *hmox-1* $^{-/-}$mice seems to exhibit increased susceptibility to inflammatory condition (endotoximia, for instance) (Duckers et al. 693-98;Fujita et al. 598-604).

CO is continuously synthesized endogenously by HO-1 and HO-2. Endogenous CO produced by heme catabolism has clear physiological roles in eukaryotic cells. Both endogenous and exogenous CO seems to play an important role as an inflammatory (Wagener et al. 551-71) and anti-hyperalgesic (Steiner et al. 1673-82) agent by relatively undetermined physiological mechanisms. Yet, acting in the CNS CO arising from HO may play an important role in fever generation (Steiner, Colombari, and Branco R499-R507).

Actually, regulation of the HO activity is of clinical interest. For instance, down-regulating HO activity has been shown to be therapeutically important to solve the problem of jaundice (also known as *icterus*) in newborns — especially those infants born in deprived socioeconomic settings who had a particular risk of brain damage resulting from uncontrolled hyperbilirubinemia (Kappas, Munson, and Marshall 1374-77). According to Abraham and Kappas (Abraham and Kappas 79-127) that HO inducers will also have important applications in various diseases, such as hypertension, diabetes, and cardiovascular disease.

The amount of data reported for the HO pathway is not as extensive as for NO pathway. This section discusses briefly some biochemical characteristics of the HO pathway as well as highlights the few existing data about HO as an interesting enzyme responsible for temperature regulation.

In mammals, biliverdin is then converted to bilirubin by the cytosolic enzyme biliverdin reductase and bilirubin is then conjugated by UDP-glucuronyl transferase before being excreted into the bile. Most of the bilirubin formed in vivo is derived from hemoglobin released from aging or damaged erythrocytes (Schacter 349-69). In culture, a number of cell types (hepatic, renal, testicular, brain, etc) catalyze heme degradation to biliverdin (Abraham et al. 543-&).

HO was first purified to homogeneity from rat liver (Maines, Ibrahim, and Kappas 5900-03). It is now clear that two isoenzymes of HO exist i.e., the original enzyme was designated

HO-1 and the second isoenzyme was designated HO-2. These isoenzymes are the products of two distinct genes, but share approximately 40% amino acid sequence homology (Mccoubrey and Maines 155-61). HO-1 is the product of only one transcript, but HO-2 is encoded by two transcripts from one gene (Mccoubrey and Maines 155-61). HO-2 is constitutively expressed, whereas HO-1 is inducible by a large number of structurally unrelated pharmacological and other agents as well as by a variety of circumstances, such as inflammation and other forms of cellular stress.

HO-1 activity can be increased in whole animal tissues by treating the animals with its natural substrate heme, as well as other stimuli such as cytochines and LPS.

The role of HO-2 in cells is not well understood. Perhaps, HO-2 may play a role in epidermal cells, germ cell development, and signal transduction in neural tissues (Abraham et al. 543-&).

Carbon Monoxide

Two major sources of CO in biological systems have been reported, one is HO-dependent, and the other is HO-independent, i.e., due to the photo-oxidation and the auto-oxidation of organic molecules, phenols, and flavenoids and the peroxidation of lipids as a result of severe stress, which may not be achieved under physiological conditions (Rodgers et al. 2-10). However, the fast increase of CO that take place in vivo is only due to the induction of HO (either HO-1 or HO-2) (Ibraham, Friedland, and Levere 75-130). Because the major source of endogenous produced CO is the degradation of heme by HO, it is now clear that CO may work as an important cellular signal molecule. It is interesting to note that NOS is a heme containing enzyme. It has been proposed that some NO effects can be duplicated by CO, including action of certain neurotransmitters could be regulated by both molecules (Abraham and Kappas 79-127). Such interaction between NO and CO may be responsible for thermoregulatory mechanisms which seems to be a fairly ripe research area for scientists interested in thermoregulation.

Evidence exist that CO, similarly to the NO, activates soluble sGC leading to a rise in cyclic (GMP) levels, which may account for a number of its physiological effects ((Maines, Ibrahim, and Kappas 5900-03). Endogenous CO arises from the cleavage of the heme molecule yielding biliverdin, free iron and CO, a process catalyzed by the enzyme heme oxygenase (HO) (Abraham and Kappas 79-127). Three distinct HO isoforms encoded by different genes have been identified, among which HO-1 and HO-2 are the best studied and known. HO-2 is constitutively expressed throughout the body, including in the CNS, whereas HO-1 is sparsely found in other tissues (Abraham et al. 543-&), but may be overexpressed in response to a series of stimuli, including hypoxia (Panchenko, Farber, and Korn C92-C101).

Steiner et al (Steiner and Branco 263-88;Steiner, Colombari, and Branco R499-R507) have provided evidence suggesting that CO plays thermoregulatory actions by acting in the CNS, including hypoxia-induced anapyrexia (Paro, Steiner, and Branco 339-43). Its pyrogenic effect has been latter confirmed by others (Jang et al. 343-48). CO seems to exert a key function to prevent excessive decreases in T_c. This may be of particular importance if we

take into consideration that the HO–CO pathway is the first pyretic pathway to be reported to play a role in anapyrexia.

Actually, ischemia (which is accompanied by a reduced tissue oxygen deliver) has been found to induce increased expression of HO-1 mRNA in the rat brain (Takeda et al. 120-24). As to other tissues, hypoxia is known to worsen heart diseases, causing a pressure overload to the right ventricle. The analysis of the distribution of HO-1 in heart tissue shows that the level of HO-1 in the atrium and right ventricle are higher than those in the left ventricle (Abraham et al. 73-81). To study the role of HO in the pathogenesis during this pressure overload to the right ventricle, rats were exposed to a hypoxia (10% O_2) and HO-1 mRNA measured. There is an increase in both the right and left ventricles within 1 h of exposure to hypoxia. Maximal HO mRNA levels are observed after 3 days and remain high for 14 days in the right ventricle, whereas left ventricle levels returned to basal levels by the 7th day of the exposure to hypoxia (Tanaka et al. 8-14). It has been postulated that the HO may be induced as a protective mechanism in the heart, as it has been observed that CO, a product of HO activity, relaxes coronary and aortic smooth muscles (Maines 389-97). One can speculate that HO pathway may improve cardiac function during hypoxia by means of dilatation of the coronary arteries. Prostaglandin synthesis (PGA) is also stimulated in the heart muscle in response to ischemia and PGA is known to be a potent inducer of HO expression in myoblastic cells (Rossi and Santoro 455-63), which implies that induction of HO by PGA in myoblasts may be a protective mechanism during hypoxia exposure.

4. Anapyrexia: regulated hypothermia

A drop in Tb is also beneficial during hypoxia exposure because, among other effects, it reduces oxygen demand when oxygen availability is limited. Consistent with this notion, evidence has been reported supporting the occurrence of a regulated reduction in Tb, just like the opposite of fever. A term to this phenomenon was suggested: anapyrexia (Cabanac, 1987). As it is defined in the Glossary of Terms for Thermal Physiology ([Anon] 75-106), anapyrexia (Gk. ana—reverse, pyretos—fever) is a pathological condition in which there is a regulated decrease in Tb, distinct from hypothermia in that thermoregulatory responses indicate a defence of the lower level of Tb. Although a recent review (Romanovsky R992-R995) suggested that this term is not suitable because this response seems to be incompatible with a single set-point model of Tb control and has a strong dependence on ambient temperature, it is clear that if many animal species exposed to a variety of hostile stimuli, have the chance to decrease their Tb, they will, and the outcome of this will be improving their survival (Steiner, Rocha, and Branco R1412-R1422;Steiner and Branco 263-88).

In 1943, Fay reported a beneficial effect of hypothermia in septic patients (Fay 1109). Now a days, hypothermia has been still referred as beneficial in certain clinical settings such as acute brain injury (Jordan and Carhuapoma 35-38). Considering that anapyrexia is a regulated response, the mechanisms underling this phenomenon may give insights to improve therapeutic hypothermia that usually requires pharmacologic intervention to blunt thermoregulatory defences, such as intense vasoconstriction and vigorous shivering. These responses are likely to be injurious to patients since they may be accompanied by

hypertension, tachycardia and activation of sympathetic nervous system cf. (Bicego, Barros, and Branco 616-39).

Hypoxia comprises the anapyretical stimulus most studied and reviewed (Wood 71-85;Wood 1249-56;Steiner and Branco 0263-88). We will emphasise new data provided by literature.

Physiological conditions may generate a reduction in oxygen availability for living organisms such as exposure to a hypoxic environments (reduced O2 partial pressure), such as altitude, burrows and oxygen-deprived water habitats (Nilsson and Renshaw 3131-39). And regarding pathologies, obstructive sleep apnea and chronic obstructive pulmonary disease are examples of conditions in which patients suffer from hypoxia (Reissmann et al. 410-16).

It is well established that hypoxia-induced anapyrexia occurs in animals raging phylogenetically from protozoan to mammals (Wood 71-85). Among mammals, hypoxic-anapyrexia has been extensively studied in laboratory rats, mice, hamsters and guinea pigs cf (Bicego, Barros, and Branco 616-39). The data obtained is consistent with the notion that hypoxia-induced anapyrexia is a beneficial response due to a decreased metabolic rate, an improved oxygen extraction in the lungs, attenuated energetic costly responses like hyperventilation and increased cardiac output, inhibited thermogenesis, increased heat loss, and survival rates, preserved brain ATP levels, and shifted thermoneutral zone to lower temperatures (Bicego, Barros, and Branco 616-39;Wood 71-85;Steiner and Branco 263-88). Barros et al (Barros et al. 603-12) compared the thermoneutral zone during normoxia and hypoxia in the Canadian golden-mantled ground squirrels and emphasized that the Tb drop induced by hypoxia represents a regulated hypothermia.

Both in vitro and in vivo toxicity of many environmental chemicals and drugs like heavy metals, methylmercury, pesticides and ethanol is directly proportional to temperature (Gordon 81-89). Moreover, there is evidence that hypothermia induced by intoxication is beneficial to survival since the lethality of most toxic agents increases with rising (Gordon et al. 161-78). It seems not to be known if toxic agents-induced hypothermia is mediated by gaseous neurotransmitters.

Mechanisms (mediators, nuclei and pathways)

Considering the whole thermoregulatory system, including sensors, afferent pathways, central nervous system nuclei that process the thermal information, efferent pathways, effectors and also the behavioral component, we can say that the mechanisms responsible for anapyrexia remain poorly understood. The best known phenomenon is hypoxic-anapyrexia, so we will attempt to focus on this issue.

We believe the hypoxic reduction of Tb is a regulated response (Steiner and Branco 263-88;Tattersall and Milsom 33-42) rather than a lack of control (Gautier et al. 2477-84) to low oxygen availability. The drop in Tb is consistent with a downward resetting of the thermoregulatory set point or with a reduction in the thermal balance. Thus the thermoregulatory sites in the central nervous system may be signaled to drive the adequate effectors—decreasing heat production and increasing heat loss.

It is interesting to note that, the anaerobic metabolism end products lactate (most vertebrates) (Bicego et al. 810-15) and ethanol (goldfish) (Shoubridge and Hochachka 165-95) arise as potential mediators of hypoxic anapyrexia. At least in mammals, when an inhibitor of acid lactic production, dichloroacetate, was used to directly address the question of whether lactate mediates the drop in Tb induced by hypoxia or not, the results were negative (Bicego et al. 810-15), i.e., lactate does not seem to be a mediator of hypoxia-induced anapyrexia in rats. Further research is needed to firmly settle this matter. Regarding the other anaerobic end product, in the goldfish Carassius auratus (family Cyprinidae), ethanol immersion or intrahypothalamic administration decrease preferred Tb (Crawshaw, Wollmuth, and Oconnor R133-R137).

Other substances have been tested and suggested to be involved or not in the development of hypoxic anapyrexia. This issue is the focus of previous reviews (Branco, Gargaglioni, and Barros 82-89;Steiner and Branco 263-88;Wood 71-85). More recently, an intracellular signaling in the most important thermointegrative region of the central nervous system, the POA, involving the second messengers, cAMP and cGMP, has been proposed as the neurochemical model (Steiner, Rocha, and Branco R1412-R1422) that includes the mediators nitric oxide, serotonin (5-HT) and dopamine (Branco, Gargaglioni, and Barros 82-89). Steiner et al. (Steiner, Rocha, and Branco R1412-R1422) reported that during hypoxia exposure, 5-HT and nitric oxide elicit a concomitant activation of cAMP and cGMP pathways in the POA, respectively, increasing intracellular levels of these two second messengers. This might cause an elevation in the thermal sensitivity of preoptic warm-sensitive neurons (Wright et al. R1704-R1715) leading to inhibition of thermogenesis and activation of heat loss, and finally resulting in Tb reduction, as recently reported.

Evidence indicates that, in addition to their role in nociceptive and stress responses, endogenous opioids are involved in thermoregulation during hypoxia (Mayfield and Dalecy 683-88;Steiner and Branco 263-88), as well as during euthermia (Mayfield et al. R1615-R1622).

Besides the effect of the above mentioned agents which decrease Tb during hypoxia, it has been suggested that there are agents that counteract this effect, possibly preventing excessive decreases in Tb (Paro, Steiner, and Branco 339-43). Indeed, as mentioned before the endogenously produced gas carbon monoxide acting in the central nervous system has been reported to play a counter-regulatory effect during hypoxia-induced anapirexia (Paro, Steiner, and Branco 339-43). Thus, as described for the febrile response, anapyrexia would result from a balance between contrasting influences of anapyretic inductors (nitric oxide, serotonin, dopamine, adenosine) and inhibitors (carbon monoxide).

5. Conclusions

The existing data about the neurochemistry responsible for temperature regulation are far from enough to provide a clear scenario. More experimental data remains urgently needed. Recent reports have recently added important details about the afferent pathways to the brain signalling during fever (Blatteis 194-223). Based on the present article it may be evident that

the tools (from biochemistry, pharmacology and genetics, mainly regarding NO) are available for testing their effect on thermoregulation.

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Reference List

Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. Pharmacological Reviews. 2008; 60(1):79–127. [PubMed: 18323402]

Abraham NG, et al. The Physiological Significance of Heme Oxygenase. International Journal of Biochemistry. 1988; 20(6):543. [PubMed: 3292310]

Abraham NG, et al. Identification of Heme Oxygenase and Cytochrome-P-450 in the Rabbit Heart. Journal of Molecular and Cellular Cardiology. 1987; 19(1):73–81. [PubMed: 3550106]

Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochemical Journal. 2001; 357:593–615. [PubMed: 11463332]

Balon TW, Nadler JL. Evidence that nitric oxide increases glucose transport in skeletal muscle. Journal of Applied Physiology. 1997; 82(1):359–63. [PubMed: 9029239]

Barros RCH, et al. Hypoxic metabolic response of the golden-mantled ground squirrel. Journal of Applied Physiology. 2001; 91(2):603–12. [PubMed: 11457771]

Beavo JA, Conti M, Heaslip RJ. Multiple Cyclic-Nucleotide Phosphodiesterases. Molecular Pharmacology. 1994; 46(3):399–405. [PubMed: 7935318]

Bellamy TC, Garthwaite J. The receptor-like properties of nitric oxide-activated soluble guanylyl cyclase in intact cells. Molecular and Cellular Biochemistry. 2002; 230(1–2):165–76. [PubMed: 11952092]

Bian K, et al. The nature of heme/iron-induced protein tyrosine nitration. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100(10):5712–17. [PubMed: 12709594]

Bicego KC, Barros RCH, Branco LGS. Physiology of temperature regulation: Comparative aspects. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology. 2007; 147(3): 616–39.

Bicego KC, et al. Is lactate a mediator of hypoxia-induced anapyrexia? Pflugers Archiv- European Journal of Physiology. 2002; 444(6):810–15. [PubMed: 12355182]

Bilban M, et al. Heme oxygenase and carbon monoxide initiate homeostatic signaling. Journal of Molecular Medicine-Jmm. 2008; 86(3):267–79.

Blatteis CM. Endotoxic fever: New concepts of its regulation suggest new approaches to its management. Pharmacology & Therapeutics. 2006; 111(1):194–223. [PubMed: 16460809]

Branco LGS, Gargaglioni LH, Barros RCH. Anapyrexia during hypoxia. Journal of Thermal Biology. 2006; 31(1–2):82–89.

Campos AH, Calixto JB. Mechanisms Involved in the Contractile Responses of Kinins in Rat Portal-Vein Rings - Mediation by B-1 and B-2 Receptors. Journal of Pharmacology and Experimental Therapeutics. 1994; 268(2):902–09. [PubMed: 8114004]

Cannon B, Nedergaard J. Brown adipose tissue: Function and physiological significance. Physiological Reviews. 2004; 84(1):277–359. [PubMed: 14715917]

Crawshaw LI, Wollmuth LP, Oconnor CS. Intracranial Ethanol and Ambient Anoxia Elicit Selection of Cooler Water by Goldfish. American Journal of Physiology. 1989; 256(1):R133–R137. [PubMed: 2912205]

Csiszar A. Structural and functional diversity of adaptor proteins involved in tyrosine kinase signalling. Bioessays. 2006; 28(5):465–79. [PubMed: 16615089]

Currie DA, de Vente J, Moody WJ. Developmental appearance of cyclic guanosine monophosphate (cGMP) production and nitric oxide responsiveness in embryonic mouse cortex and striatum. Developmental Dynamics. 2006; 235(6):1668–77. [PubMed: 16518821]

Dias MB, et al. Role of nitric oxide in tolerance to lipopolysaccharide in mice. Journal of Applied Physiology. 2005; 98(4):1322–27. [PubMed: 15579566]

Ding Z, et al. Icilin induces a hyperthermia in rats that is dependent on nitric oxide production and NMDA receptor activation. European Journal of Pharmacology. 2008; 578(2–3):201–08. [PubMed: 17976579]

Duckers HJ, et al. Heme oxygenase-1 protects against vascular constriction and proliferation. Nature Medicine. 2001; 7(6):693–98.

Elhusseini AED, Bladen C, Vincent SR. Molecular Characterization of A Type-Ii Cyclic Gmp-Dependent Protein-Kinase Expressed in the Rat-Brain. Journal of Neurochemistry. 1995; 64(6): 2814–17. [PubMed: 7760063]

Erusalimsky JD, Moncada S. Nitric oxide and mitochondrial signaling from physiology to pathophysiology. Arteriosclerosis Thrombosis and Vascular Biology. 2007; 27(12):2524–31.

Fay T. Cooling in Shock. Journal of the American Medical Association. 1943; 121:1109.

Feleder C, Perlik V, Blatteis CM. Preoptic norepinephrine mediates the febrile response of guinea pigs to lipopolysaccharide. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2007; 293(3):R1135–R1143.

Fujita T, et al. Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. Nature Medicine. 2001; 7(5):598–604.

Furchgott RF, Zawadzki JV. The Obligatory Role of Endothelial-Cells in the Relaxation of Arterial Smooth-Muscle by Acetylcholine. Nature. 1980; 288(5789):373–76. [PubMed: 6253831]

Garthwaite J. Concepts of neural nitric oxide-mediated transmission. European Journal of Neuroscience. 2008; 27(11):2783–802. [PubMed: 18588525]

Garthwaite J. Concepts of neural nitric oxide-mediated transmission. European Journal of Neuroscience. 2008; 27(11):2783–802. [PubMed: 18588525]

Garthwaite J. Concepts of neural nitric oxide-mediated transmission. European Journal of Neuroscience. 2008; 27(11):2783–802. [PubMed: 18588525]

Gautier H, et al. Hypoxia-Induced Changes in Shivering and Body-Temperature. Journal of Applied Physiology. 1987; 62(6):2477–84. [PubMed: 3610940]

Gerassimou C, et al. Regulation of the expression of soluble guanylyl cyclase by reactive oxygen species. British Journal of Pharmacology. 2007; 150(8):1084–91. [PubMed: 17339839]

Gibb BJ, Garthwaite J. Subunits of the nitric oxide receptor, soluble guanylyl cyclase, expressed in rat brain. European Journal of Neuroscience. 2001; 13(3):539–44. [PubMed: 11168561]

Gonzalez D, Drapier JC, Bouton C. Endogenous nitration of iron regulatory protein-1 (IRP-1) in nitric oxide-producing murine macrophages - Further insight into the mechanism of nitration in vivo and its impact on IRP-1 functions. Journal of Biological Chemistry. 2004; 279(41):43345–51. [PubMed: 15258160]

Gordon CJ. The therapeutic potential of regulated hypothermia. Emergency Medicine Journal. 2001; 18(2):81–89. [PubMed: 11300205]

Gordon CJ, et al. Temperature Regulation in Laboratory Mammals Following Acute Toxic Insult. Toxicology. 1988; 53(2–3):161–78. [PubMed: 3062847]

Hofmann F, Biel M, Kaupp UB. International Union of Pharmacology. XLII. Compendium of voltage-gated ion channels: Cyclic nucleotide-modulated channels. Pharmacological Reviews. 2003; 55(4): 587–89. [PubMed: 14657416]

Ibraham NG, Friedland ML, Levere RD. Heme Metabolism in Erythroid and Hepatic Cells. Progress in Hematology. 1983; 13:75–130. [PubMed: 6366915]

Ilagan RP, et al. Differences in a conformational equilibrium distinguish catalysis by the endothelial and neuronal nitric-oxide synthase flavoproteins. Journal of Biological Chemistry. 2008; 283:28.

Jang CG, et al. Carbon monoxide as a novel central pyrogenic mediator. Archives of Pharmacal Research. 2002; 25(3):343–48. [PubMed: 12135108]

Jian KH, et al. Nitric oxide modulation of voltage-gated calcium current by S-nitrosylation and cGMP pathway in cultured rat hippocampal neurons. Biochemical and Biophysical Research Communications. 2007; 359(3):481–85. [PubMed: 17544367]

Jordan JD, Carhuapoma JR. Hypothermia: Comparing technology. Journal of the Neurological Sciences. 2007; 261(1–2):35–38. [PubMed: 17532342]

Jurado S, Sanchez-Prieto J, Torres M. Differential expression of NO-sensitive guanylyl cyclase subunits during the development of rat cerebellar granule cells: regulation via N-methyl-D-aspartate receptors. Journal of Cell Science. 2003; 116(15):3165–75. [PubMed: 12799420]

Kappas A, Munson DP, Marshall JR. Sn-mesoporphyrin interdiction of severe hyperbilirubinemia in Jehovah's witness newborns as an alternative to exchange transfusion. Pediatrics. 2001; 108(6): 1374–77. [PubMed: 11731664]

Kellogg DL, Zhao JL, Wu Y. Neuronal nitric oxide synthase control mechanisms in the cutaneous vasculature of humans in vivo. Journal of Physiology-London. 2008; 586(3):847–57.

Kloss S, Srivastava R, Mulsch A. Down-regulation of soluble guanylyl cyclase expression by cyclic AMP is mediated by mRNA-stabilizing protein HuR. Molecular Pharmacology. 2004; 65(6):1440–51. [PubMed: 15155837]

Kobzik L, et al. Nitric-Oxide in Skeletal-Muscle. Nature. 1994; 372(6506):546–48. [PubMed: 7527495]

Kozak W, Kozak A. Genetic models in applied physiology - Selected Contribution: Differential role of nitric oxide synthase isoforms in fever of different etiologies: studies using Nos gene-deficient mice. Journal of Applied Physiology. 2003; 94(6):2534–44. [PubMed: 12562678]

Lin MT, Lin JH. Involvement of tyrosine kinase in the pyrogenic fever exerted by NOS pathways in organum vasculosum laminae terminalis. Neuropharmacology. 2000; 39(2):347–52. [PubMed: 10670430]

Liu JW, Hughes TE, Sessa WC. The first 35 amino acids and fatty acylation sites determine the molecular targeting of endothelial nitric oxide synthase into the Golgi region of cells: A green fluorescent protein study. Journal of Cell Biology. 1997; 137(7):1525–35. [PubMed: 9199168]

Madhusoodanan KS, Murad F. NO-cGMP signaling and regenerative medicine involving stem cells. Neurochem Res. 2007; 32(4–5):681–94. [PubMed: 17043768]

Maines MD. Carbon-Monoxide - An Emerging Regulator of Cgmp in the Brain. Molecular and Cellular Neuroscience. 1993; 4(5):389–97. [PubMed: 19912945]

Maines MD, Ibrahim NG, Kappas A. Solubilization and Partial-Purification of Heme Oxygenase from Rat-Liver. Journal of Biological Chemistry. 1977; 252(16):5900–03. [PubMed: 18477]

Mathai ML, et al. Nitric oxide increases cutaneous and respiratory heat dissipation in conscious rabbits. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 1997; 41(6):R1691–R1697.

Mayfield KP, Dalecy LG. Delta-1 Opioid Agonist Acutely Increases Hypoxic Tolerance. Journal of Pharmacology and Experimental Therapeutics. 1994; 268(2):683–88. [PubMed: 8113979]

Mayfield KP, et al. Potential Adaptations to Acute-Hypoxia - Hct, Stress Proteins, and Set-Point for Temperature Regulation. American Journal of Physiology. 1994; 266(5):R1615–R1622. [PubMed: 8203641]

Mccoubrey WK, Maines MD. The Structure - Organization and Differential Expression of the Gene Encoding Rat Heme Oxygenase-2. Gene. 1994; 139(2):155–61. [PubMed: 8112599]

Menniti FS, Faraci WS, Schmidt CJ. Phosphodiesterases in the CNS: targets for drug development. Nature Reviews Drug Discovery. 2006; 5(8):660–70. [PubMed: 16883304]

Miguel TT, Nunes-de-Souza RL. Anxiogenic-like effects induced by NMDA receptor activation are prevented by inhibition of neuronal nitric oxide synthase in the periaqueductal gray in mice. Brain Res. 2008; 1240:39–46. [PubMed: 18793618]

Monda M, et al. Nitric-Oxide Reduces Body-Temperature and Sympathetic Input to Brown Adipose-Tissue During Pge(1)-Hyperthermia. Brain Research Bulletin. 1995; 38(5):489–93. [PubMed: 8665273]

Morishita T, et al. Nephrogenic diabetes insipidus in mice lacking all nitric oxide synthase isoforms. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(30): 10616–21. [PubMed: 16024729]

Murad F. Shattuck Lecture: Nitric oxide and cyclic GMP in cell signaling and drug development. New England Journal of Medicine. 2006; 355(19):2003–11. [PubMed: 17093251]

Nilsson GE, Renshaw GMC. Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. Journal of Experimental Biology. 2004; 207(18):3131–39. [PubMed: 15299034]

Ogiwara T, Chik CL, Ho AK. Protein kinase C potentiation of the tyrosine kinase inhibitor-stimulated cyclic GMP production in rat pinealocytes. Biochemical Pharmacology. 1997; 53(1):95–102. [PubMed: 8960068]

Ortiz PA, Garvin JL. Trafficking and activation of eNOS in epithelial cells. Acta Physiologica Scandinavica. 2003; 179(2):107–14. [PubMed: 14510773]

Panchenko MV, Farber HW, Korn JH. Induction of heme oxygenase-1 by hypoxia and free radicals in human dermal fibroblasts. American Journal of Physiology-Cell Physiology. 2000; 278(1):C92–C101. [PubMed: 10644516]

Parent A, et al. Synaptic transmission and hippocampal long-term potentiation in olfactory cyclic nucleotide-gated channel type 1 null mouse. Journal of Neurophysiology. 1998; 79(6):3295–301. [PubMed: 9636130]

Paro FM, Steiner AA, Branco LGS. Thermoregulatory response to hypoxia after inhibition of the central heme oxygenase-carbon monoxide pathway. Journal of Thermal Biology. 2001; 26(4–5): 339–43.

Perotti CAA, et al. Effects of a neuronal nitric oxide synthase inhibitor on lipopolysaccharide- induced fever. Brazilian Journal of Medical and Biological Research. 1999; 32(11):1381–87. [PubMed: 10559839]

Pollock JS, et al. Purification and Characterization of Particulate Endothelium-Derived Relaxing Factor Synthase from Cultured and Native Bovine Aortic Endothelial-Cells. Proceedings of the National Academy of Sciences of the United States of America. 1991; 88(23):10480–84. [PubMed: 1720542]

Reissmann H, et al. Resistance of laryngeal mask airway and tracheal tube in mechanically ventilated patients. British Journal of Anaesthesia. 2000; 85(3):410–16. [PubMed: 11103183]

Riccio A, et al. A nitric oxide signaling pathway controls CREB-mediated gene expression in neurons. Molecular Cell. 2006; 21(2):283–94. [PubMed: 16427017]

Rodgers PA, et al. Sources of Carbon-Monoxide (Co) in Biological-Systems and Applications of Co Detection Technologies. Seminars in Perinatology. 1994; 18(1):2–10. [PubMed: 8209283]

Romanovsky AA. Do fever and anapyrexia exist? Analysis of set point-based definitions. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2004; 287(4):R992–R995.

Rossi A, Santoro MG. Induction by Prostaglandin A(1) of Heme Oxygenase in Myoblastic Cells - An Effect Independent of Expression of the 70-Kda Heat-Shock Protein. Biochemical Journal. 1995; 308:455–63. [PubMed: 7772027]

Russwurm M, Wittau N, Koesling D. Guanylyl cyclase/PSD-95 interaction - Targeting of the nitric oxide-sensitive alpha(2)beta(1) guanylyl cyclase to synaptic membranes. Journal of Biological Chemistry. 2001; 276(48):44647–52. [PubMed: 11572861]

Ryter SW, Alam J, Choi AMK. Heme oxygenase-1/carbon monoxide: From basic science to therapeutic applications. Physiological Reviews. 2006; 86(2):583–650. [PubMed: 16601269]

Schacter BA. Heme Catabolism by Heme Oxygenase - Physiology, Regulation, and Mechanism of Action. Seminars in Hematology. 1988; 25(4):349–69. [PubMed: 3064316]

Schuman EM, Madison DV. Nitric-Oxide and Synaptic Function. Annual Review of Neuroscience. 1994; 17:153–83.

Shoubridge EA, Hochachka PW. The Integration and Control of Metabolism in the Anoxic Goldfish. Molecular Physiology. 1983; 4(3–4):165–95.

Simon E. Nitric oxide as a peripheral and central mediator in temperature regulation. Amino Acids. 1998; 14(1–3):87–93. [PubMed: 9871447]

Stamler JS, et al. (S)NO signals: Translocation, regulation, and a consensus motif. Neuron. 1997; 18(5):691–96. [PubMed: 9182795]

Steiner AA, Antunes-Rodrigues J, Branco LGS. Role of preoptic second messenger systems (cAMP and cGMP) in the febrile response. Brain Research. 2002; 944(1–2):135–45. [PubMed: 12106673]

Steiner AA, et al. Antipyretic role of the NO-cGMP pathway in the anteroventral preoptic region of the rat brain. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2002; 282(2):R584–R593.

Steiner AA, Branco LG. Fever and anapyrexia in systemic inflammation: intracellular signaling by cyclic nucleotides. Front Biosci. 2003; 8:s1398–s1408. [PubMed: 12957844]

Steiner AA, Branco LGS. Hypoxia-induced anapyrexia: Implications and putative mediators. Annual Review of Physiology. 2002; 64:263–88.

Steiner AA, et al. Role of the haeme oxygenase/carbon monoxide pathway in mechanical nociceptor hypersensitivity. British Journal of Pharmacology. 2001; 132(8):1673–82. [PubMed: 11309238]

Steiner AA, Colombari E, Branco LGS. Carbon monoxide as a novel mediator of the febrile response in the central nervous system. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 1999; 277(2):R499–R507.

Steiner AA, Rocha MJA, Branco LGS. A neurochemical mechanism for hypoxia-induced anapyrexia. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2002; 283(6):R1412–R1422.

Steiner AA, et al. Febrigenic signaling to the brain does not involve nitric oxide. British Journal of Pharmacology. 2004; 141(7):1204–13. [PubMed: 15006900]

Sulakhe PV, et al. Guanylate Cyclase - Subcellular-Distribution in Cardiac-Muscle, Skeletal- Muscle, Cerebral-Cortex and Liver. Biochemical Journal. 1976; 157(3):705–12. [PubMed: 10890]

Sullivan BM, Wong S, Schuman EM. Modification of hippocampal synaptic proteins by nitric oxide-stimulated ADP ribosylation. Learning & Memory. 1997; 3(5):414–24. [PubMed: 10456108]

Takeda A, et al. Increased Expression of Heme Oxygenase Messenger-Rna in Rat-Brain Following Transient Forebrain Ischemia. Brain Research. 1994; 666(1):120–24. [PubMed: 7889361]

Tanaka T, et al. Pharmacogenomics of cardiovascular pharmacology: Pharmacogenomic network of cardiovascular disease models. Journal of Pharmacological Sciences. 2008; 107(1):8–14. [PubMed: 18490853]

Tattersall GJ, Milsom WK. Transient peripheral warming accompanies the hypoxic metabolic response in the golden-mantled ground squirrel. Journal of Experimental Biology. 2003; 206(1):33–42. [PubMed: 12456695]

Tegeder I, et al. Reduced inflammatory hyperalgesia with preservation of acute thermal nociception in mice lacking cGMP-dependent protein kinase I. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(9):3253–57. [PubMed: 14973199]

Tenhunen R, Marver HS, Schmid R. Enzymatic Conversion of Heme to Bilirubin by Microsomal Heme Oxygenase. Proceedings of the National Academy of Sciences of the United States of America. 1968; 61(2):748. [PubMed: 4386763]

Uhler MD. Cloning and Expression of A Novel Cyclic Gmp-Dependent Protein-Kinase from Mouse-Brain. Journal of Biological Chemistry. 1993; 268(18):13586–91. [PubMed: 8514791]

Wagener FADT, et al. Different faces of the heme-heme oxygenase system in inflammation. Pharmacological Reviews. 2003; 55(3):551–71. [PubMed: 12869663]

Weight FF, Petzold G, GREENGAR P. Guanosine 3′,5′-Monophosphate in Sympathetic-Ganglia - Increase Associated with Synaptic Transmission. Science. 1974; 186(4167):942–44. [PubMed: 4377760]

Wood SC. Interactions Between Hypoxia and Hypothermia. Annual Review of Physiology. 1991; 53:71–85.

Wood SC. Oxygen As A Modulator of Body-Temperature. Brazilian Journal of Medical and Biological Research. 1995; 28(11–12):1249–56. [PubMed: 8728855]

Works CF, et al. Photochemical nitric oxide precursors: Synthesis, photochemistry, and ligand substitution kinetics of ruthenium salen nitrosyl and ruthenium salophen nitrosyl complexes. Inorganic Chemistry. 2002; 41(14):3728–39. [PubMed: 12099878]

Wright CL, et al. Cyclic GMP alters the firing rate and thermosensitivity of hypothalamic neurons. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2008; 294(5):R1704–R1715.

Wright CL, et al. Cyclic GMP alters the firing rate and thermosensitivity of hypothalamic neurons. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2008; 294(5):R1704–R1715.

Wyckoff MH, et al. Plasma membrane estrogen receptors are coupled to endothelial nitric-oxide synthase through G alpha(i). Journal of Biological Chemistry. 2001; 276(29):27071–76. [PubMed: 11369763]

Zamboni G, et al. The capacity to accumulate cyclic AMP in the preoptic-anterior hypothalamic area of the rat is affected by the exposition to low ambient temperature and the subsequent recovery. Experimental Brain Research. 1996; 109(1):164–68. [PubMed: 8740221]

Glossary of terms for thermal physiology - Third edition (Reprinted from the Japanese Journal of Physiology). Journal of Thermal Biology. 2003; 28(1):75–106.