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Yawning: role of hypothalamic paraventricular nitric oxide

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KEY WORDS yawning; nitric oxide; nitric oxide donors; nitric-oxide synthase; paraventricular hypothalamic nucleus; dopamine agonists; oxytocin; *N*-methylaspartate; calcium; narcotics

ABSTRACT

Yawning is a phylogenetically old, stereotyped event that occurs alone or associated with stretching and/or penile erection in humans, in animals from reptiles to birds and mammals, under different conditions. Several neurotransmitters and neuropeptides are involved in its control at the central level. One of these at the level of the paraventricular hypothalamic nucleus (PVHN) is nitric oxide (NO). First, NO synthase inhibitors injected into this hypothalamic nucleus prevent yawning induced by dopamine agonists, oxytocin or *N*-methyl-*D*-aspartic acid (NMDA), which induce yawning by activating PVHN oxytocinergic neurons projecting to extrahypothalamic brain areas. The inhibitory effect of NO synthase inhibitors was not observed when these compounds were given concomitantly with *L*-arginine, the precursor of NO. Second, dopamine agonists, NMDA and oxytocin given at doses that induce yawning, increase NO production in the PVHN, as determined by *in vivo* microdialysis. Conversely, the opiate morphine, which prevents yawning induced by dopamine agonists, oxytocin and NMDA, also prevents the increase in the paraventricular NO production induced by these compounds. Third, NO donors, such as nitroglycerin, sodium nitroprusside and hydroxylamine, induce yawning when injected into the PVHN apparently by activating oxytocinergic transmission. Since guanylate cyclase inhibitors and NO scavengers (hemoglobin) injected into the PVHN do not prevent drug-induced yawning, nor 8-Br-cGMP injected into the PVHN induces this behavioral response, it is likely that NO acts as an intracellular rather than an intercellular modulator inside the PVHN oxytocinergic neurons in which NO is formed to facilitate the expression of this phylogenetically old event by guanylate cyclase-independent mechanisms.

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INTRODUCTION

Yawning is a common physiological event that occurs with a low frequency in humans and animals. It is phylogenetically old, since it can be observed not only in mammals, but also in birds and possibly in reptiles[1,2]. It is characterized by a gaping of the mouth accompanied by a long inspiration, followed by a shorter expiration. The internal physiological stimuli that evoke spontaneous yawning and its physiological functions are unknown, although a role in increasing oxygen-CO_2 exchange in the lung, in facial stretching and in normalizing internal ear pressure has been suggested[3-5]. In humans this stereotyped behavior can be easily triggered for instance by seeing someone yawn (yawning is "contagious"), or simply by reading or thinking about it, or by being involved in boring tasks[3-5]. Its more frequent occurrence at bed and waking times and in boring situations than at other times of the day and situations, with electrophysiological findings showing that yawning occurs concomitantly with an increase in cortical electroencephalographic activity, led to the suggestion that yawning is an ancestral vestige that survived through evolution and that occurs when attention is low and arousal needs to be increased[6]. Yawning can be also observed in other contexts, for instance before eating[7], in the presence of nausea, motion sickness, brain tumors or lesions, hemorrhage and encephalitis[1,8-10], or after several neuropharmacological

manipulations (see below).

The neuropharmacological studies have revealed that the occurrence of yawning alone, but more often associated with stretching and penile erection[6,11-14] is under the control of several neurotransmitters and neuropeptides at the central level. Of these, the best known are adrenocorticotropin, α-melanocyte stimulating hormone and related peptides, acetylcholine, dopamine, serotonin, excitatory amino acids, oxytocin and opioid peptides[15]. Some of these interact at the level of the PVHN in the control of this behavioral response. In particular, it seems that dopamine receptor agonists, *N*-methyl-*D*-aspartic acid (NMDA) and oxytocin itself induce yawning by activating oxytocinergic neurons originating in this hypothalamic nucleus and projecting to extrahypothalamic brain areas, ie, the hippocampus and/or the medulla oblongata[16], while the opiate morphine prevents the behavioral response induced by the above agents by inhibiting the oxytocinergic neurons mediating yawning[17]. In contrast, these oxytocinergic neurons seem not involved in the yawning induced by adrenocorticotropin peptides[18,19] or by serotonin receptor agonists[20,21]. Most important, the PVHN is one of the richest brain areas containing nitric-oxide (NO) synthase[22], the Ca^{2+}-calmodulin-dependent iron containing enzyme responsible for the formation of NO, a novel discovered neurotransmitter/neuromodulator[23-28]. Detailed immunocytochemical studies have shown that the enzyme in the PVHN is localized in cell bodies and perikarya of neurons containing oxytocin, vasopressin, and somatostatin[23,29-31] often close to neurons containing corticotropin-releasing hormone or luteinizing hormone-releasing hormone, suggesting that NO in the PVHN might be involved in the release of these releasing factors[32]. The localization of a Ca^{2+}-dependent NO synthase in PVHN oxytocinergic neurons[31,33] in close proximity with dopamine[34,35], serotonin[36], and excitatory amino acid projections[37] and the above mentioned involvement of NO in the release of corticotropin-releasing hormone that controls the release of adrenocorticotropin, raised the possibility that NO was involved in the control of yawning at the level of the PVHN. This short review summarizes published and unpublished work that shows that paraventricular NO has a physiological role in the control of this phylogenetically old event.

NO SYNTHASE INHIBITORS PREVENT DRUG- AND NEUROPEPTIDE-INDUCED YAWNING WHEN INJECTED INTO THE PVHN

The involvement of NO in the control of yawning was first suggested by studies showing that NO synthase inhibitors prevent this response when induced by dopamine agonists, oxytocin and NMDA in male rats. In these studies two well characterized competitive inhibitors, N^G-nitro-*L*-arginine methyl ester and *N*-monomethyl-*L*-arginine but not its inactive isomer *N*-monomethyl-*D*-arginine[38] were found able to prevent apomorphine-, oxytocin- and NMDA-induced yawning in a dose-dependent manner when injected into a lateral ventricle. The potency of these compounds in preventing yawning was correlated with their potency in inhibiting NO synthase, being N^G-nitro-*L*-arginine methyl ester 4 - 5 times more potent than *N*-monomethyl-*L*-arginine and *N*-mono methyl-*D*-arginine was ineffective[39,40]. It is soon shown that the PVHN is one brain area where NO synthase inhibitors acted to prevent the yawning induced by the above compounds. Indeed N^G-nitro-*L*-arginine methyl ester prevented the drug-induced response when injected into this hypothalamic nucleus but not in surrounding structures or in other brain areas thought to be involved in the control of yawning[40,41]. That N^G-nitro-*L*-arginine methyl ester prevention of drug-induced yawning is mediated by the competitive inhibition of NO synthase and not by other effects of this compound, was further supported by the ability of *L*-arginine, the physiological substrate of NO synthase, to prevent the inhibitory effect of N^G-nitro-*L*-arginine methyl ester, although given at a dose unable to induce *per se* yawning (Fig 1)[42].

NO DONORS INDUCE YAWNING BY ACTIVATING OXYTOCINERGIC NEURONS IN THE PVHN

The above results are in line with the hypothesis that NO is involved in the regulation of yawning induced by dopamine agonists, oxytocin and NMDA at

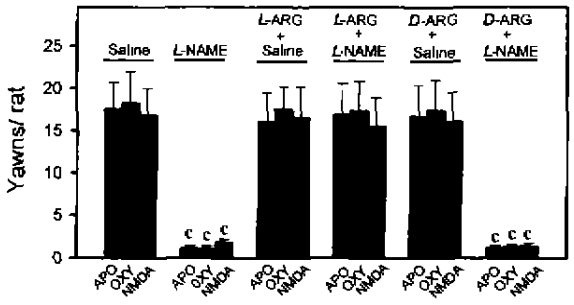

Fig 1. Apomorphine-, oxytocin-, and NMDA-induced yawning is prevented by *L*-NAME injected into the PVHN: reversal by *L*-arginine. Rats implanted with a chronic guide cannula directed to the PVHN and/or to the lateral ventricles were placed individually in a Plexiglas cage. Apomorphine (sc 80 μg·kg^{-1}), oxytocin (30 ng into the PVHN), and NMDA (50 ng into the PVHN) were given after a 30-min habituation period. Saline (0.3 μL), *L*-NAME (20 μg) alone or together with *L*-arginine (50 μg) or *D*-arginine (50 μg) were given into the PVHN 15 min before apomorphine, oxytocin or NMDA in a volume of 0.3 μL. After treatment, the animals were placed in the Plexiglass cage and observed for 60 min in order to count yawning episodes. Values are means ± SEM of 7 rats found to have the the tip of the probe in the PVHN as determined by histological analysis. $^cP < 0.01$ *vs* the values of the corresponding groups treated with apomorphine, oxytocin or NMDA (one-way ANOVA followed by Duncan's multiple range test).

the PVHN level. This hypothesis is further supported by the findings showing that NO donors injected icv or directly into the PVHN induce yawning episodes indistinguishable from those induced by the agents mentioned above. Of these, the most effective are nitroglycerin, sodium nitroprusside, hydroxylamine, and isoamyl nitrite. Nitroglycerin induces yawning when injected into the lateral ventricle (icv) or into the PVHN, isoamyl nitrite when injected icv, sodium nitroprusside and hydroxylamine only when injected into the PVHN[43,44]. Interestingly, *L*-arginine, the physiological precursor of NO, was ineffective when injected into the lateral ventricle, but induced yawning when injected into the PVHN in more than 70 % of the treated rats. This response was not observed with *D*-arginine[42].

As to the mechanism by means of which NO donors induce yawning when injected into the PVHN, it is likely that these compounds increase central oxytocinergic transmission, as already shown for oxytocin, dopamine agonists and NMDA. In agreement with this hypothesis, yawning induced by nitroglycerin, sodium nitroprusside, hydroxylamine or isoamyl nitrite, like that induced by dopamine agonists, oxytocin and NMDA, is prevented by the administration into the lateral ventricle of the potent oxytocin receptor antagonist [d $(CH_2)_5$-Tyr $(Me)^2$-Orn^8]-vasotocin[45] given into the lateral ventricle but not in the PVHN (Fig 2)[42,43].

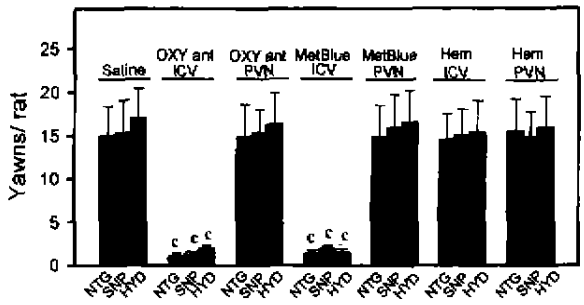

Fig 2. NO donors induce yawning when injected into the PVHN: effect of [d $(CH_2)_5$Tyr $(Me)^2$-Orn^8]-vasotocin, methylene blue and hemoglobin given icv or into the PVHN. Nitroglycerin (NTG, 6 μg), sodium nitroprusside (SNP, 20 μg) and hydroxylamine (HYD, 20 μg) were injected into the PVHN in a volume of 0.3 μL. [d $(CH_2)_5$Tyr $(Me)^2$-Orn^8]-vasotocin (OXY ant, 0.1 μg into the PVHN or 1 μg icv), methylene blue (MetBlue, 20 μg into the PVHN or 400 μg icv) and hemoglobin (Hem, 20 μg into the PVHN or 400 μg icv) were given into the PVHN in a volume of 0.3 μL or icv in a volume of 10 μl 15 min before the NO donors. The animals were placed individually in a Plexiglass cage and observed for 60 min to count yawning episodes. values are the means ± SEM of 6 rats per group. $^cP < 0.01$ with respect to the corresponding group of NO donor-treated rats (one-way ANOVA followed by Duncan's multiple range test).

Thus, it seems reasonable to assume that yawning induced by NO donors injected into the PVHN is also mediated by the activation of the same oxytocinergic neurons projecting to extrahypothalamic brain areas, which are activated by dopamine agonists, NMDA and oxytocin (see Introduction).

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DOPAMINE AGONISTS, OXYTOCIN, AND NMDA INDUCE YAWNING BY ACTIVATING NO SYNTHASE IN THE PVHN

Together, the ability of NO synthase inhibitors to prevent apomorphine-, oxytocin-, and NMDA-induced yawning and the ability of NO donors to induce yawning when injected into the PVHN is in line with the hypothesis that apomorphine, oxytocin, and NMDA induce yawning by activating NO synthase in the PVHN. Support for this possibility was obtained by measuring NO production in the PVHN *in vivo*. This was achieved by measuring the concentration of the reaction products of newly formed NO with O_2 and H_2O, NO_2^- and NO_3^-, which represent an indirect but reliable indicator of NO production *in vivo*[25,46-48], in the dialysate collected from a vertical microdialysis probe implanted in the PVHN, after the administration of the above substances. Apomorphine, oxytocin or NMDA, given at a dose that induces yawning, all increased the baseline concentration of NO_2^- four – six-fold (Fig 3) and of NO_3^- two-fold in the paraventricular dialysate. This increase was correlated with the increase in the number of yawning episodes, was observed already 20 min after the treatment that induced yawning and lasted for 40 – 80 min (Fig 3). In agreement with a key role of PVHN neurons in this response, the increase in NO_2^- and NO_3^- concentration was found only when the tip of the dialysis probe was found located in the PVHN, but not in surrounding structures, as determined by histological analysis[47,49,50]. The drug-induced increase of NO production was reasonably due to the activation of NO synthase secondary to the stimulation of dopamine, oxytocin or NMDA receptors. Indeed, NO_2^- increase was prevented by haloperidol, a classical dopamine receptor antagonist, when it was induced by apomorphine, by the oxytocin receptor antagonist [*d* $(CH_2)_5$Tyr (Me)^2-Orn^8]-vasotocin given into the PVHN, when it was induced by oxytocin, or by the antagonist of NMDA receptor antagonist dizocilpine, when it was induced by NMDA. Conversely, in all cases NO_2^- increase was prevented by N^G-nitro-*L*-arginine methyl ester, injected into the lateral ventricle 15 min before the above compounds, which also prevented yawning (Fig 3)[47,49,50].

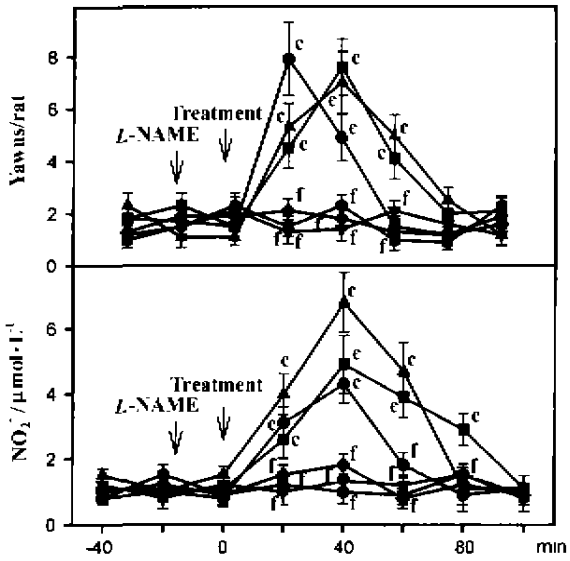

Fig 3. Apomorphine, NMDA, and oxytocin induce yawning and increase NO_2^- concentration in the paraventricular dialysate obtained with a vertical microdialysis probe implanted in the PVHN of freely moving male rats: prevention by *L*-NAME. Rats implanted with a vertical microdialysis probe directed to the PVHN and/or a cannula for icv microinjections or into the PVHN were placed individually in a Plexiglas cage and perfused with a Ringer solution. Treatments (apomorphine, sc 80 μg·kg^{-1}, circles; NMDA, 50 ng into the PVHN, triangles; and oxytocin, 30 ng into the PVHN, squares) were given after a 120-min equilibration period of the probe with the perfusion buffer (time = 0). *L*-NAME (20 μg) was given into the PVHN 15 min before apomorphine (rhombs), NMDA (updown triangles) or oxytocin (hexagons) in a volume of 0.3 μL. The perfusion rate was 2 μL/min. Aliquots of 40 μL were collected every 20 min and analyzed for NO_2^- content[47,49,50]. During the perfusion, rats were observed to count yawning episodes. Each value is the mean ± SEM of 6 rats found to have the the tip of the probe in the PVHN as determined by histological analysis. $^cP < 0.01$ with respect to pretreatment values (negative times). $^fP < 0.01$ with respect to the values of the corresponding group treated with apomorphine, NMDA or oxytocin (one-way ANOVA followed by Duncan's multiple range test).

As to the mechanism by means of which apomorphine, oxytocin or NMDA activate NO synthase in the PVHN, one possibility is that these compounds increase Ca^{2+} influx in the cell bodies of oxytocinergic neurons mediating yawning. In this regard it is pertinent to recall that NMDA receptors are coupled

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with voltage-dependent Ca^{2+} channels[51] that once activated by NMDA lead to an increased Ca^{2+} influx, which in turn activates the Ca^{2+}-calmodulin-dependent NO synthase[50]. It is likely that dopamine and oxytocin receptors also are coupled to a G protein-dependent transduction system that increases Ca^{2+} influx[52,53] (for a review of the transduction systems coupled with dopamine receptors and oxytocin receptors), since apomorphine- and oxytocin-induced yawning is prevented by pertussis toxin[54], which inactivates several G proteins, and by ω-conotoxin GVIA[55], a potent blocker of *N*-type Ca^{2+} channels[56]. In agreement with the above findings, ω-conotoxin microinjected into the PVHN not only prevents yawning induced by oxytocin or apomorphine, but also the NO_2^- (Fig 4) and NO_3^- increase, which occurs concomitantly to yawning in the paraventricular dialysate of rats treated with the above substances[57]. However, ω-conotoxin does not prevent NMDA-induced NO production and yawning. This suggest that ω-conotoxin-sensitive Ca^{2+} channels play only a minor role, if any, in the NMDA-induced activation of NO synthase in paraventricular oxytocinergic neurons mediating yawning. This is in line with the hypothesis recalled to above that NMDA effects are mediated by NMDA receptor-coupled Ca^{2+} channels[50]. Accordingly, ω-conotoxin is also found unable to prevent NMDA-induced NO production in cultured striatal neurons[58]. ω-Conotoxin also does not prevent yawning induced by NO donors sodium nitroprusside and hydroxylamine when they are injected into the PVHN[57]. This suggests that NO formed by NO donors does not influence Ca^{2+} influx in the cell bodies of oxytocinergic neurons mediating yawning at the PVHN level. This finding is also in line with the hypothesis discussed below that NO formed by these compounds (and endogenous NO as well) acts as intracellular messanger inside the oxytocinergic neurons mediating yawning[57].

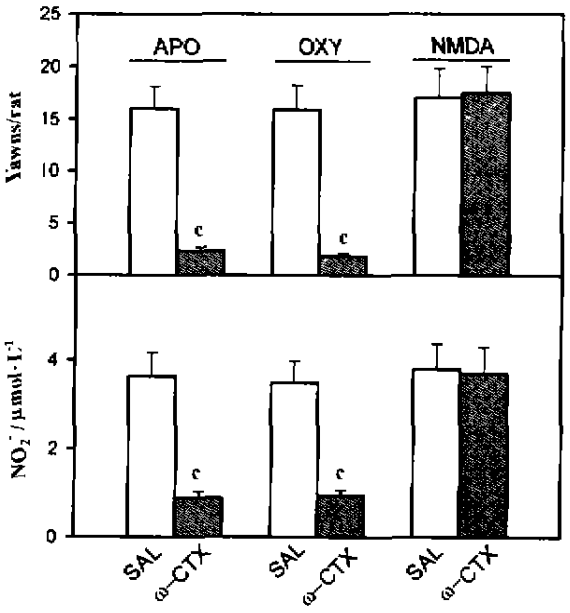

Fig 4. Effect of ω-conotoxin on apomorphine-, oxytocin-, and NMDA-induced yawning and the concomitant NO_2^- concentration increase that occurs in the paraventricular dialysate. Rats implanted with a vertical microdialysis probe directed at the PVHN were used. The experimental conditions were identical to those reported in the legend of Fig 3. Saline, apomorphine (sc 80 μg·kg^{-1}), oxytocin (30 ng into the PVHN), and NMDA (50 ng into the PVHN) were given after a 120-min equilibration period of the probe with the perfusion buffer. Saline (0.3 μL) or ω-conotoxin (5 ng) were given into the PVHN 10 min before apomorphine, oxytocin or NMDA. Each value is the mean ± SEM of 6 rats found to have the the tip of the probe in the PVHN as determined by histological analysis. NO_2^- values are those measured 20 – 40 min after the treatment (see also Fig 3). $^cP < 0.01$ with respect to the values of the corresponding group treated with apomorphine, oxytocin or NMDA (One-way ANOVA followed by Duncan's multiple range test).

MORPHINE PREVENTS YAWNING INDUCED BY DOPAMINE RECEPTOR AGONISTS, NMDA AND OXYTOCIN BY A MECHANISM INVOLVING PARAVENTRICULAR NO

The studies cited above show that dopamine, excitatory amino acids, and oxytocin facilitate the expression of yawning, apparently by increasing NO production in the cell bodies of paraventricular oxytocinergic neurons, causing in turn an increase of central oxytocinergic transmission. There is also evidence that NO is also involved in the prevention of the yawning induced by apomorphine, oxytocin, and NMDA by the opiate morphine injected into the PVHN. In this regard, it is pertinent to recall first that the

PVHN contains endogenous opioid peptides and receptors[59], which exert an inhibitory control on oxytocinergic transmission[60-62], second, that morphine, which stimulates opioid receptors, is very effective to prevent the yawning induced by dopamine receptor agonists, oxytocin, and NMDA, when injected into the PVHN[17,63], and third that yawning is one of the most common signs of the opiate withdrawal syndrome[64]. Accordingly, morphine injected into the PVHN prevents apomorphine-, oxytocin-, and *N*-methyl-*D*-aspartic induced yawning, and this occurs concomitantly with a prevention of the increase in paraventricular NO production induced by the above compounds, as measured by *in vivo* microdialysis (Fig 5)[63,65]. The morphine prevention of NO production induced by the above compounds is prevented by naloxone, an opioid receptor antagonist, and it is apparently mediated by the stimulation of opioid receptors of the μ type, since U-69593, an opioid receptor agonist 500 times more potent than morphine on the opioid receptors of the k type, is ineffective in preventing either yawning or the increase in NO production induced by apomorphine, oxytocin, and NMDA (Fig 5)[63,65]. The prevention by morphine of the increase of NO production induced by the above compounds could result from a decreased Ca^{2+} influx in the cell bodies of oxytocinergic neurons mediating the behavioral response that, in turn, decreases NO synthase activity, as discussed above for the prevention of apomorphine- or oxytocin-induced responses by ω-conotoxin and of NMDA-induced response by dizocilpine. Although the molecular mechanisms by means of which stimulation of μ opioid receptors causes a decreased Ca^{2+} influx are unknown, the finding suggests that opioids and NO have opposite effects on the expression of yawning at the paraventricular level.

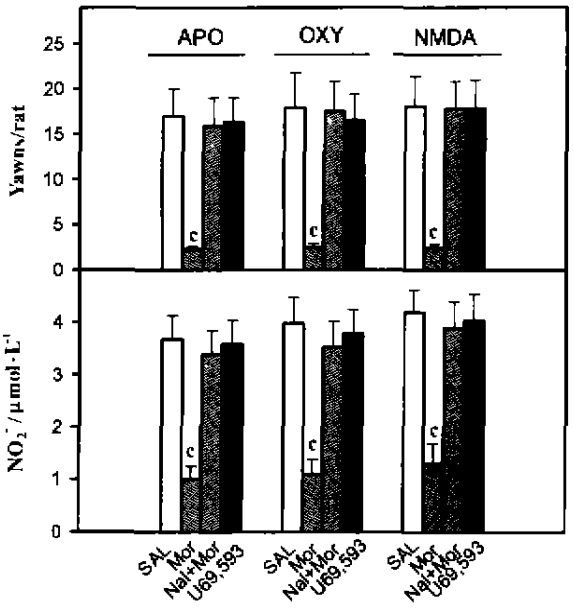

Fig 5. Prevention by morphine but not by U-69593 of apomorphine-, oxytocin-, and NMDA-induced yawning and NO_2^- concentration increase in the paraventricular dialysate obtained with a vertical microdialysis probe implanted in the PVHN of freely moving male rats: reversal by naloxone. Rats implanted with a vertical microdialysis probe directed at the PVHN were used. The experimental conditions were identical to those reported in the legend of Fig 3. Saline, apomorphine (sc 80 μg · kg^{-1}), oxytocin (30 ng into the PVHN), and NMDA (50 ng into the PVHN) were given after a 120-min equilibration period of the probe with the perfusion buffer. Saline (0.3 μL), morphine (5 μg) and U-69593 (10 μg) were given into the PVHN 15 min before apomorphine, oxytocin or NMDA. Naloxone (10 μg into the PVHN) was given 15 min before morphine. Each value is the mean ± SEM of 6 rats found to have the the tip of the probe in the PVHN as determined by histological analysis. NO_2^- values are those measured 20 – 40 min after the treatment (see also Fig 3). $^cP < 0.01$ with respect to the values of the corresponding group treated with apomorphine, oxytocin or NMDA (One-way ANOVA followed by Duncan's multiple range test).

DOES NO ACTIVATE GUANYLATE CYCLASE IN THE PVHN TO FACILITATE YAWNING?

The studies summarized above suggest that either endogenous NO formed by the stimulation of dopamine, NMDA or oxytocin receptors in the PVHN or by NO donors injected into the PVHN, activates oxytocinergic transmission to induce yawning. However, these results do not reveal the mechanism by means of which NO activates oxytocinergic neurons in the PVHN. One of the best known targets of NO in peripheral tissues and in several brain areas is guanylate cyclase[25-28,66]. Nevertheless, from the results available so far guanylate cyclase seems not to be the target of NO responsible for the induction of yawning at least in the PVHN. Indeed, methylene blue and 6-

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(phenylamino)-5,8-quinolinedione, two putative inhibitors of guanylate cyclase[67,68] injected into the PVHN are unable to prevent yawning induced by apomorphine, oxytocin, NMDA and NO donors as well[40,41,43]. Methylene blue injected into the PVHN is also unable to prevent the increase in NO production induced by apomorphine, oxytocin, and NMDA in the paraventricular dialysate (Fig 6)[47,49,50]. In this regard it is pertinent to recall that NO might interact with many other enzymes that, like guanylate cyclase, bind metal ions such iron, and that other targets of NO, such as cellular adenosine diphosphate-ribosyl-transferases, have been identified[27] (for a review of the targets of NO see 27). However this interpretation is complicated by the ability of both methylene blue and 6-(phenylamino)-5,8-quinolinedione injected into the lateral ventricle to prevent yawning induced by apomorphine, oxytocin, NMDA and NO donors (Fig 2)[40,41,43]. One possible explanation for this discrepancy is that cyclic guanosine 3′:5′-monophosphate is involved in the expression of yawning induced by the above substances in some yet undiscovered brain area distant from the PVHN. In agreement with this possibility, methylene blue given into the PVHN is unable to prevent the increase of NO production in the PVHN induced by apomorphine, oxytocin, and NMDA (Fig 6) despite its ability to prevent yawning when injected into the lateral ventricle (Fig 2). That guanylate cyclase might not be the target of NO in the PVHN for the induction of yawning is also suggested by the finding that 8-bromo-cyclic guanosine 3′:5′-monophosphate, a stable cyclic guanosine 3′:5′-monophosphate analog that would be expected to mimic the effect of endogenous cyclic guanosine 3′:5′-monophosphate, is unable to induce yawning when injected into the PVHN in male rats[43]. Although a NO-cyclic guanosine 3′:5′-monophosphate signalling pathway has been well characterized not only by biochemical but also by immunocytochemistry studies in other brain areas, such as the hippocampus and the cerebellum[23,28,66], such pathway might not occur in the PVHN. In agreement with this hypothesis, the PVHN contains only very low amounts of guanylate cyclase immunoreactivity[66] and PVHN oxytocinergic neurons, labelled by NO synthase-directed antibodies, are not labelled by guanylate cyclase-directed antibodies[31]

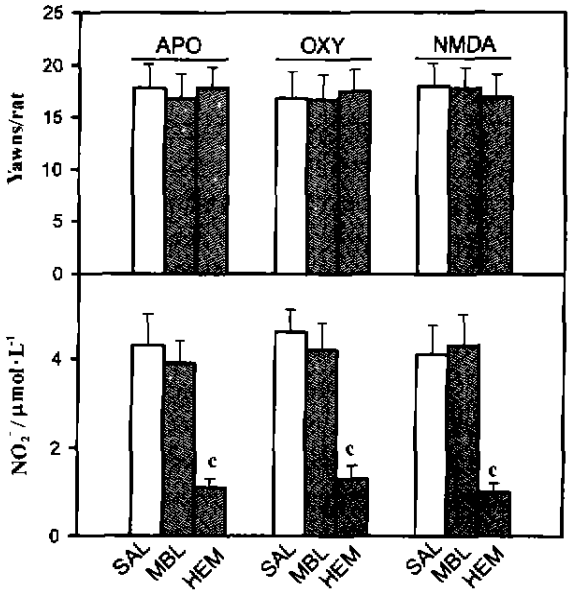
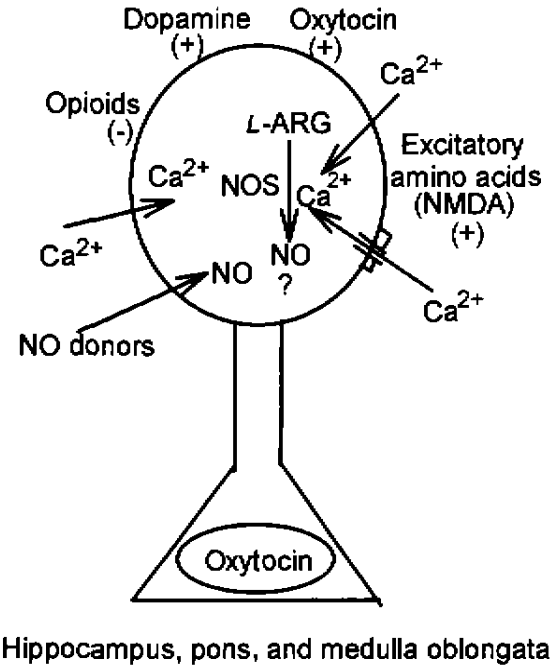


Fig 6. Apomorphine, oxytocin, and NMDA induce yawning and increase NO_2^- concentration in the paraventricular dilaysate obtained with a vertical microdialysis probe implanted in the PVHN of freely moving male rats: effect of methylene blue and hemoglobin. Rats implanted with a vertical microdialysis probe directed to the PVHN equiped with a cannula for microinjections into the PVHN were placed individually in a Plexiglas cage and perfused with a Ringer solution as described in the legend of Fig 3. Apomorphine (sc 80 μg·kg⁻¹), oxytocin (30 ng into the PVHN), and NMDA (50 ng into the PVHN) were given after a 120-min equilibration period of the probe with the perfusion buffer (time = 0). Saline (0.3 μL), methylene blue (20 μg), and hemoglobin (20 μg) were given into the PVHN 15 min before apomorphine, oxytocin or NMDA in a volume of 0.3 μL. Each value is the mean ± SEM of 6 rats found to have the tip of the probe in the PVHN as determined by histological analysis. NO_2^- values are those measured 20 – 40 min after the treatment (see also Fig 3). $^cP < 0.01$ with respect to the values of the corresponding group treated with apomorphine, oxytocin or NMDA (One-way ANOVA followed by Duncan's multiple range test).

NO ACTS AS AN INTRACELLULAR MESSENGER TO FACILITATE YAWNING

Hemoglobin, a potent NO scavenger[69], injected either into the lateral ventricle or in the PVHN is unable

to prevent yawning induced by oxytocin, apomorphine, NMDA or NO donors (Fig 2 and Fig 6)[40,41,43]. Nevertheless, hemoglobin injected into the PVHN is able to prevent the increase in NO production induced by dopamine receptor agonists, oxytocin or NMDA (Fig 6)[47,49,50]. Since hemoglobin would bind NO exclusively in the extracellular space, being unable to cross cellular membranes because of its high molecular weight[67], the finding suggests that NO is acting intracellularly in those neurons in which is formed to induce yawning. However, this does not rule out the possibility that NO might act as an intercellular messenger in the paraventricular nucleus. Indeed NO released out from the PVHN neurons where it is formed and scavenged by hemoglobin might mediate other dopamine, oxytocin or NMDA effects in close PVHN neurons or other hypothalamic structures[32] (for a review of the functions of NO in the hypothalamus).



CONCLUDING REMARKS

The above results suggest a key role of NO in the control of yawning at the paraventricular level. NO might be synthetized by NO synthase in the cell bodies of oxytocinergic neurons projecting to extrahypothalamic brain areas mediating this behavioral response. Accordingly, NO synthase is activated by agents supposed to induce yawning by acting on these neurons in the PVHN, possibly by increasing Ca^{2+} influx in their cell bodies (Fig 7). The activation of NO synthase is necessary for the induction of the behavioral response by dopamine agonists, oxytocin or NMDA, since the response does not occur when the enzyme has been previously inhibited, for instance by NO synthase inhibitors injected into the PVHN. Once formed, NO activates a yet unidentified target, apparently different from guanylate cyclase, which in turn leads to the activation of oxytocinergic neurons, which mediate the expression of this behavioral response by releasing oxytocin in sites distant from the PVHN, ie the hippocampus and/or the medulla oblongata[16,43,47,49,50]. Interestingly, NO seems to act as an intracellular rather than an intercellular messenger, since the behavioral response is not prevented by NO scavenging from the extracellular space[47,49,50].

Finally, although the studies reviewed above show that paraventricular NO plays a key role in the control

Fig 7. Schematic representation of a hypothetical mechanism of action by means of which paraventricular oxytocinergic neurons projecting to extrahypothalamic brains areas mediate yawning induced by several neurotransmitters and/or neuropeptides. According to this model, the activation of these neurons by dopamine, excitatory amino acids and oxytocin itself causes yawning, while their inhibition by opioid peptides, at least when activated by the above compounds, inhibits the behavioral response. Dopamine, oxytocin, and excitatory amino acids activate these neurons the first two by stimulating specific receptors coupled to a pertussis toxin-sensitive G protein that leads to the opening of ω-conotoxin-sensitive Ca^{2+} channels, and the third by stimulating Ca^{2+} channel-coupled NMDA receptors. This would cause a influx of Ca^{2+} ions that would act as a second messenger, and would activate the Ca^{2+}-calmodulin-dependent NO synthase. NO formed endogenously (or derived by NO donors) would activate in turn an yet undiscovered c-GMP-independent processes, inside the cell bodies of the oxytocinergic neurons that lead to their activation, thereby releasing oxytocin at sites distant from the PVHN, ie the hippocampus, the pons and/or the medulla oblongata. The mechanism by means of which opioids inhibit oxytocinergic transmission is still unknown. However, evidence showing that opioid receptors might be located in the paraventricular oxytocinergic cell bodies and that their activation prevents the activation of NO synthase by dopamine, oxytocin, and excitatory amino acids have been provided (see text for details).

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of yawning, it is pertinent to recall that NO might be involved in the control of this response also in sites different from the PVHN. Among these are the yet undiscovered brain areas in which NO synthase inhibitors given into the lateral ventricle but not in the PVHN act to prevent yawning induced by serotonin receptor agonists of the 5-HT_{2c} type[20] or by adrenocorticotropin-related peptides[20]. Since a possible site of action of 5-HT_{2c} receptor agonists is the medulla oblongata[21], and NO synthase has been identified in this structure[71,72], this area may be another brain site in which NO is involved in the expression of yawning.

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呵欠：下丘脑旁室一氧化氮的作用

R971.2 R

Meli., MR

关键词 呵欠；一氧化氮；一氧化氮供体；一氧化氮合酶；下丘脑室旁核；多巴胺激动剂；缩宫素；*N*-甲基精氨酸；钙；麻醉品

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