

Melis, M. R. and A. Argiolas (1999). "Yawning: role of hypothalamic paraventricular nitric oxide." *Zhongguo Yao Li Xue Bao* 20(9): 778-88.

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 extra-hypothalamic 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.

Melis, M. R., S. Succu, et al. (2000). "EP 60761 and EP 50885, two hexarelin analogues, induce penile erection in rats." *Eur J Pharmacol* 404(1-2): 137-43.

The effect of hexarelin and four related peptide analogues, EP 40904, EP 40737, EP 50885 and EP 60761, injected into the paraventricular nucleus of the hypothalamus of male rats in doses between 2 and 2000 ng on spontaneous penile erection was studied. Of these peptides, EP 60761 and EP 50885, but not hexarelin, EP 40904 or EP 40737, increased dose-dependently the number of spontaneous penile erections. EP 60761 was active already at the dose of 20 ng, which induced the sexual response in 70% of the treated rats. The maximal response was induced by 200 ng of the peptide. EP 50885 was less potent than EP 60761, with 1000 ng being the minimal effective dose and 2000 ng as the dose required to induce the maximal response. At the doses used, both peptides also increased slightly the number of spontaneous yawning episodes. EP 60761- and EP 50885-induced penile erection was prevented by the oxytocin receptor antagonist [d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)<sub>2</sub>-Orn<sub>8</sub>]vasotocin (0.1-1 microg) given intracerebroventricularly (*i.c.v.*), but not into the paraventricular nucleus (0.1-1 microg), by the competitive nitric oxide (NO) inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) given either into the paraventricular nucleus (10-20 microg) or *i.c.v.* (75-150 microg), by the N-type Ca<sup>2+</sup> channel blocker omega-conotoxin-GVIA (2-5 ng) or by the opiate morphine (1-10 microg), but not by the dopamine receptor antagonist (Z)-4-[3-[2-(trifluoromethyl)-9H-thioxanthen-9-ylidene]propyl]-1-pipecazine-ethanol (*cis*-flupenthixol) (10 microg) or by the N-methyl-D-aspartic acid (NMDA) receptor antagonist (5R, 10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine ((+)-MK-801) (1 microg), all given into the paraventricular nucleus before either peptide. The present results show that EP 60761 and EP 50885 induced penile erection by increasing central oxytocin transmission, possibly by activating NO synthase in

the cell bodies of oxytocinergic neurons located in the paraventricular nucleus that control penile erection.

Argiolas, A., M. R. Melis, et al. (2000). "ACTH- and alpha-MSH-induced grooming, stretching, yawning and penile erection in male rats: site of action in the brain and role of melanocortin receptors." *Brain Res Bull* 51(5): 425-31.

The effect of adrenocorticotropin (ACTH)(1-24) and alpha-melanocyte stimulating hormone (alpha-MSH) on grooming, stretching, yawning and penile erection was studied after injection into different brain areas. Both peptides induce the above responses when injected into the hypothalamic periventricular region of the third ventricle. This region includes the paraventricular nucleus, the dorsomedial nucleus, the ventromedial nucleus and the anterior hypothalamic area. The minimal effective dose of both peptides was 0.5 microg and the maximal effect was seen with 2 microg, the highest dose tested. Irrespective of the injection site, grooming started 5-7 min after injection of either peptide, while stretching, yawning and penile erection started only after 15-35 min and lasted for 90-120 min. In contrast both peptides were ineffective when injected into the preoptic area, the caudate nucleus or the CA1 field of the hippocampus. Grooming, stretching and yawning, but not penile erection, were prevented by cyclic[AcCys(11), D-Nal(14), Cys(18), AspNH(2)(22)]-beta-MSH (11-22) (HS014), a selective melanocortin 4 receptor antagonist, injected into the same periventricular area 10 min before of ACTH(1-24) or alpha-MSH. The results show that ACTH(1-24) and alpha-MSH act in the hypothalamic periventricular region to induce the above responses and that grooming, stretching and yawning, but not penile erection, are mediated by melanocortin 4 receptors.

Rosaria Melis, M., M. S. Spano, et al. (2000). "Activation of gamma-aminobutyric acid(A) receptors in the paraventricular nucleus of the hypothalamus reduces apomorphine-, N-methyl-D-aspartic acid- and oxytocin-induced penile erection and yawning in male rats." *Neurosci Lett* 281(2-3): 127-30.

The effect of muscimol and baclofen injected into the paraventricular nucleus of the hypothalamus on penile erection and yawning induced by apomorphine, oxytocin and N-methyl-D-aspartic acid (NMDA) was studied in male rats. Muscimol (20-200 ng), but not baclofen (200 ng), injected into the paraventricular nucleus of the hypothalamus 10 min before apomorphine (50 ng), oxytocin (10 ng) or NMDA (50 ng) reduced penile erection and yawning induced by the above compounds given into the paraventricular nucleus. Bicuculline (250 ng) injected into the paraventricular nucleus 5 min before muscimol (100 ng) prevented the inhibitory effect of muscimol on penile erection and yawning induced by apomorphine, oxytocin and NMDA. The present results show that gamma-aminobutyric acid (GABA) inhibits penile erection and yawning by acting on GABA(A) receptors in the paraventricular nucleus of the hypothalamus.

Succu, S., M. S. Spano, et al. (1998). "Different effects of omega-conotoxin on penile erection, yawning and paraventricular nitric oxide in male rats." *Eur J Pharmacol* 359(1): 19-26.

A dose of apomorphine or oxytocin that induces penile erection and yawning increases nitric oxide production in the paraventricular nucleus of the hypothalamus, as determined by the increase in NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> concentration induced by these substances in the paraventricular dialysate obtained from male rats. All the above responses were prevented by a dose of omega-conotoxin-GVIA as low as 5 ng. This potent inhibitor of N-type Ca<sup>2+</sup> channels was injected into the paraventricular nucleus 15 min before apomorphine (50 ng) or oxytocin (10 ng). In contrast, omega-conotoxin was ineffective when the above responses were

induced by N-methyl-D-aspartic acid (50 ng). The peptide toxin (5 ng) was also ineffective on the penile erection and yawning induced by the nitric oxide donors sodium nitroprusside (50 microg) or hydroxylamine (50 microg), injected into the paraventricular nucleus. The present results suggest that omega-conotoxin-sensitive Ca<sup>2+</sup> channels are involved in the activation of nitric oxide synthase, penile erection and yawning induced by apomorphine and oxytocin, but not by N-methyl-D-aspartic acid, at the paraventricular level.

Melis, M. R., S. Succu, et al. (1997). "Prevention by morphine of N-methyl-D-aspartic acid-induced penile erection and yawning: involvement of nitric oxide." *Brain Res Bull* 44(6): 689-94.

The effect of morphine on the increase of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> concentration in the dialysate obtained with a microdialysis probe implanted in the paraventricular nucleus of the hypothalamus, and penile erection and yawning induced by N-methyl-D-aspartic acid (NMDA) was studied in male rats. NMDA (50 ng) injected in the paraventricular nucleus of the hypothalamus, induced penile erection and yawning and increased NO<sub>2</sub><sup>-</sup> from 1.10 +/- 0.28 microM to 7.30 +/- 1.10 microM and NO<sub>3</sub><sup>-</sup> from 5.05 +/- 0.71 microM to 11.03 +/- 1.61 microM. Morphine (1-10 microg), but not U-69,593 (10 microg), a selective agonist of the kappa opiate receptor subtype, prevented in a dose-dependent manner NMDA-induced increase in NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> concentration when injected in the paraventricular nucleus 15 min before NMDA. Morphine prevention of NMDA-induced NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> increase was related to a concomitant decrease in the number of penile erection and yawning episodes induced by the excitatory amino acid. Morphine effect was not observed in male rats treated with the opiate receptor antagonist naloxone (10 microg) microinjected in the paraventricular nucleus 15 min before morphine. The present results suggest that morphine prevents an NMDA-induced increase in paraventricular NO production, penile erection, and yawning by inhibiting NO synthase activity in the paraventricular nucleus of the hypothalamus through the stimulation of opioid receptors of the micro subtype.

Melis, M. R. and A. Argiolas (1997). "Role of central nitric oxide in the control of penile erection and yawning." *Prog Neuropsychopharmacol Biol Psychiatry* 21(6): 899-922.

1. Recent experimental evidence has shown that nitric oxide (NO) plays an important role in the expression of penile erection and yawning and that this molecule has to be added to the list of the best known neurotransmitters and neuropeptides involved in this symptomatology.
2. This was first suggested by the ability of NO synthase inhibitors injected in the lateral ventricles (i.c.v.) or in the paraventricular nucleus of the hypothalamus (PVN) to prevent these behavioral responses induced by dopamine agonists, oxytocin and NMDA. The inhibitory effect of NO synthase inhibitors was not observed when these compounds were injected concomitantly with L-arginine, the precursor of NO. Most important, this hypothalamic nucleus is one of the richest brain areas of NO synthase and also the brain site where dopamine, NMDA and oxytocin act to induce penile erection and yawning by activating central NO synthase containing oxytocinergic neurons.
3. NO synthase inhibitors given i.c.v. but not in the PVN prevent also penile erection and yawning induced by ACTH and serotoninlc agonists, which induce these responses by acting with mechanisms unrelated to oxytocinergic transmission.
4. Dopamine agonists, NMDA and oxytocin increase NO production in the PVN at doses that induce penile erection and yawning, as determined by measuring the concentration of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> in the dialysate obtained with a vertical probe implanted in the PVN by in vivo microdialysis.
5. NO donors, such as nitroglycerin, sodium nitroprusside and hydroxylamine, induce

penile erection and yawning indistinguishable from those induced by oxytocin, dopamine agonists or NMDA when injected in the PVN. The NO donor response was prevented by the i.c.v. injection of the oxytocin receptor antagonist d(CH<sub>2</sub>)<sub>5</sub>-Tyr(Me)-Orn<sup>8</sup>-vasotocin, indicating that these compounds also induce penile erection and yawning by activating oxytocinergic transmission. 6. Finally, guanylate cyclase inhibitors (i.e. methylene blue and LY 83583) and hemoglobin injected in the PVN do not prevent rug-induced penile erection and yawning, nor 8-Br-cGMP injected in the PVN induces these behavioral responses suggesting that the mechanism by means of which endogenous or NO donor-derived NO facilitates oxytocinergic transmission to induce penile erection and yawning is not related to the activation of guanylate cyclase. Furthermore, since hemoglobin, in spite of its ability to prevent drug-induced NO production in the PVN, does not prevent penile erection and yawning, it is likely that NO acts as an intracellular rather than an intercellular modulator in the PVN neurons in which is formed to facilitate the expression of these behavioral responses.

Melis, M. R., S. Succu, et al. (1997). "N-methyl-D-aspartic acid-induced penile erection and yawning: role of hypothalamic paraventricular nitric oxide." *Eur J Pharmacol* 328(2-3): 115-23.

A dose of N-methyl-D-aspartic acid (NMDA, 50 ng) that induces penile erection and yawning when injected into the paraventricular nucleus of the hypothalamus, increased the concentration of NO<sub>2</sub><sup>-</sup> from 1.10 +/- 0.28 microM to 7.32 +/- 1.12 microM and of NO<sub>3</sub><sup>-</sup> from 4.96 +/- 0.69 microM to 10.5 +/- 1.61 microM in the paraventricular dialysate obtained from male rats by in vivo microdialysis. NO<sub>2</sub><sup>-</sup> concentration was not increased by (+/-)-alpha-(amino)-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 100 ng) or by trans-(+/-)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) (100 ng), which were unable to induce these behavioral responses. N-Methyl-D-aspartic acid effect on NO<sub>2</sub><sup>-</sup> concentration, penile erection and yawning was prevented by dizolcipine (MK-801) (10-100 ng) or by the nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester (20 microg), but not by the oxytocin receptor antagonist [d(CH<sub>2</sub>)<sub>5</sub>,Tyr(Me)<sub>2</sub>,Orn<sup>8</sup>]vasotocin (100 ng), or by the guanylate cyclase inhibitor methylene blue (20 microg) given in the paraventricular nucleus 15 min before N-methyl-D-aspartic acid or by the dopamine receptor antagonist haloperidol (0.5 mg/kg) given intraperitoneally 30 min before N-methyl-D-aspartic acid. In contrast, the nitric oxide scavenger hemoglobin (20 microg) given in the paraventricular nucleus prevented N-methyl-D-aspartic acid-induced NO<sub>2</sub><sup>-</sup> concentration increase, but was unable to prevent penile erection and yawning. The results suggest that N-methyl-D-aspartic acid induces penile erection and yawning by increasing nitric oxide synthase activity in the paraventricular nucleus of the hypothalamus, possibly in the cell bodies of oxytocinergic neurons projecting to extra-hypothalamic brain areas and mediating these behavioral responses.

Melis, M. R., S. Succu, et al. (1997). "Prevention by morphine of apomorphine- and oxytocin-induced penile erection and yawning: involvement of nitric oxide." *Naunyn Schmiedeberg's Arch Pharmacol* 355(5): 595-600.

The possible involvement of nitric oxide in the prevention by morphine of apomorphine- and oxytocin-induced penile erection and yawning was investigated by measuring the concentration of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> in the dialysate obtained with a vertical microdialysis probe implanted in the paraventricular nucleus of the hypothalamus of male rats. Either apomorphine (80 micrograms/kg s.c.) or oxytocin (30 ng i.c.v.) increased significantly basal NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> concentration in the paraventricular dialysate, penile erection and yawning.

Morphine (1.5 and 10 mg/kg i.p.) prevented dose-dependently either apomorphine or oxytocin responses when given 15 min before apomorphine or oxytocin. Prevention by morphine of apomorphine and oxytocin responses was abolished by naloxone (3 mg/kg i.p.) given 15 min before morphine. Morphine prevented apomorphine and oxytocin responses also when given in the lateral ventricles or directly in the paraventricular nucleus. In contrast, the selective agonist of the kappa opioid receptor subtype U-69,593 was found to be ineffective. The present results confirm previous findings showing that morphine acts through mu receptors in the paraventricular nucleus to prevent apomorphine and oxytocin-induced penile erection and yawning and suggest that this morphine effect is mediated by a decreased activity of nitric oxide in the paraventricular nucleus of the hypothalamus.

Melis, M. R., S. Succu, et al. (1997). "Oxytocin increases nitric oxide production in the paraventricular nucleus of the hypothalamus of male rats: correlation with penile erection and yawning." *Regul Pept* 69(2): 105-11.

A dose of oxytocin (50 ng i.c.v.) that induces penile erection and yawning, increased the concentration of NO<sub>2</sub><sup>-</sup> from 0.98 +/- 0.29 to 4.2 +/- 0.79 microM and of NO<sub>3</sub><sup>-</sup> from 5.6 +/- 0.33 to 12.03 +/- 0.99 microM in the dialysate from the paraventricular nucleus of the hypothalamus of male rats, as measured by in vivo microdialysis. NO<sub>2</sub><sup>-</sup> concentration was also increased by [Thr<sup>4</sup>, Gly<sup>7</sup>]-oxytocin (100 ng i.c.v. and oxytocin(8) (1 microgram i.c.v.) which also induced penile erection and yawning, but not by oxytocin(1-6) (1 microgram i.c.v.) or oxytocin (7-9) 1 microgram i.c.v.), which were unable to induce these behavioral responses. The oxytocin effect on NO<sub>2</sub> concentration, penile erection and yawning was prevented by the oxytocin receptor antagonist, d(CH<sub>2</sub>)<sub>5</sub>,Tyr(Me)-Orn<sup>8</sup>-vasotocin (1 microgram i.e.v.) or by the nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (200 micrograms i.c.v.), but not by the dopamine receptor antagonist, haloperidol (0.5 mg/kg i.p.). The nitric oxide scavenger, hemoglobin (200 micrograms i.c.v.), prevented oxytocin-induced NO<sub>2</sub><sup>-</sup> concentration increase, but was unable to prevent penile erection and yawning. Methylene blue (300 micrograms i.c.v.) an inhibitor of guanylate cyclase, was ineffective on oxytocin-induced NO<sub>2</sub><sup>-</sup> concentration increase, but prevented the behavioral responses. The results suggest that oxytocin induces penile erection and yawning by increasing nitric oxide synthase activity in the cell bodies of oxytocinergic neurons projecting to extra-hypothalamic brain areas and mediating the behavioral responses.

Melis, M. R., S. Succu, et al. (1996). "Dopamine agonists increase nitric oxide production in the paraventricular nucleus of the hypothalamus: correlation with penile erection and yawning." *Eur J Neurosci* 8(10): 2056-63.

A low dose of apomorphine (80 micrograms/kg s.c.), a mixed D<sub>1</sub>/D<sub>2</sub> agonist that induces penile erection and yawning, increased the concentration of NO<sub>2</sub><sup>-</sup> from 1.12 +/- 0.45 microM to 3.8 +/- 0.75 microM and NO<sub>3</sub><sup>-</sup> from 5.53 +/- 0.82 to 11.25 +/- 2.30 microM in the dialysate collected from the paraventricular nucleus of the hypothalamus of male rats by in vivo microdialysis. The NO<sub>2</sub><sup>-</sup> concentration was also increased by LY 171555 (50 micrograms/kg s.c.), a D<sub>2</sub> agonist that induces penile erection and yawning, but not by SKF 38393 (5 mg/kg s.c.), a D<sub>1</sub> agonist with no effect on these responses. Conversely, apomorphine's effect on NO<sub>2</sub><sup>-</sup> was prevented by haloperidol (0.5 mg/kg i.p.), a mixed D<sub>1</sub>/D<sub>2</sub> antagonist and L-sulpiride (25 mg/kg i.p.), a D<sub>2</sub> antagonist, but not by the D<sub>1</sub> agonist SCH 23390 (50 micrograms/kg s.c.), although all three compounds prevented penile erection and yawning. The apomorphine effect on NO<sub>2</sub><sup>-</sup>, penile erection and yawning was also prevented by the nitric oxide synthase inhibitor

NG-nitro-L-arginine methyl ester (200 micrograms i.c.v.). The nitric oxide scavenger haemoglobin (200 micrograms i.c.v.) also prevented the NO<sub>2</sub>-increase, but was ineffective against penile erection and yawning. In contrast, the oxytocin antagonist d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)-Orn<sub>8</sub>-vasotocin (1 microgram i.c.v.) and the guanylate cyclase inhibitor methylene blue (300 micrograms i.c.v.) had no effect on the NO<sub>2</sub>-increase, but did prevent the behavioural responses. We infer from this that dopamine agonists induce penile erection and yawning by acting on D<sub>2</sub> receptors that increase nitric oxide synthase activity in the cell bodies of paraventricular oxytocinergic neurons projecting to extra-hypothalamic brain areas.

Melis, M. R. and A. Argiolas (1995). "Nitric oxide donors induce penile erection and yawning when injected in the central nervous system of male rats." *Eur J Pharmacol* 294(1): 1-9.

In order to provide further support for a role of central nitric oxide as a mediator of penile erection and yawning, the nitric oxide donors sodium nitroprusside, hydroxylamine, isoamyl nitrite and S-nitroso-N-acetylpenicillamine were injected into the lateral ventricles (i.c.v.) or into the paraventricular nucleus of the hypothalamus of male rats. Of the above compounds injected i.c.v., only isoamyl nitrite (10-100 micrograms) induced penile erection and yawning, while the others induced dramatic behavioral changes, such as motor hyperactivity and convulsions, that masked the above responses. Nevertheless, nitric oxide donors in doses ranging from 10 to 50 micrograms, for except S-nitroso-N-acetylpenicillamine that was injected only at the dose of 10 micrograms and isoamyl nitrite that was not injected at all because of poor solubility, induced penile erection and yawning when injected in the paraventricular nucleus. Nitric oxide donor-induced responses were prevented by methylene blue and LY 83583, inhibitors of guanylate cyclase, the best known target of nitric oxide, given i.c.v. but not in the paraventricular nucleus. However, 8-bromo-guanosine 3':5'-cyclic monophosphate (8-Br-cGMP), a stable cGMP analog, and hemoglobin, a nitric oxide scavenger, were ineffective in inducing and preventing, respectively, penile erection and yawning when injected either i.c.v. or in the paraventricular nucleus. Nitric oxide donor-induced responses were also prevented by the nonapeptide oxytocin receptor antagonist d(CH<sub>2</sub>)<sub>5</sub>-Tyr(Me)-Orn<sub>8</sub>-vasotocin given i.c.v. but not in the paraventricular nucleus. The present results suggest that nitric oxide donors induce penile erection and yawning by activating central oxytocinergic transmission in the paraventricular nucleus of the hypothalamus via a cGMP-independent mechanism.

Melis, M. R., R. Stancampiano, et al. (1995). "Role of nitric oxide in penile erection and yawning induced by 5-HT<sub>1c</sub> receptor agonists in male rats." *Naunyn Schmiedeberg's Arch Pharmacol* 351(4): 439-45.

The effect of the intracerebroventricular (i.c.v.) administration of NG-nitro-L-arginine methyl ester and NG-monomethyl-L-arginine, two inhibitors of nitric oxide (NO) synthase, on penile erection and yawning induced by 1-(3-chlorophenyl)-piperazine (m-CPP)- and N-(3-trifluoromethylphenyl)-piperazine (TFMPP), two selective 5HT<sub>1c</sub> receptor agonists, was studied in male rats. Both NO synthase inhibitors (50-500 micrograms i.c.v.) prevented dose-dependently the behavioural responses induced by m-CPP (0.5 mg/kg s.c.) or by TFMPP (1 mg/kg s.c.), but NG-nitro-L-arginine methyl ester was about 4-5 times more potent than NG-monomethyl-L-arginine. The D-isomer of NG-monomethyl-L-arginine, which does not inhibit nitric oxide synthase, was ineffective. The inhibitory effect of NG-nitro-L-arginine methyl ester on m-CPP- and TFMPP-induced responses was prevented by the administration of L-arginine (1 mg i.c.v.). In contrast, NG-

nitro-L-arginine methyl ester (20 micrograms) was ineffective when injected in the paraventricular nucleus of the hypothalamus, a brain area that plays a key role in the expression of these behavioural responses. m-CPP- and TFMPP-induced penile erection and yawning was prevented also by the i.c.v. administration of LY 83583 (50-200 micrograms) or methylene blue (50-400 micrograms), two inhibitors of guanylate cyclase but not by reduced hemoglobin (50-400 micrograms), a NO scavenger. The results suggest that central nitric oxide is involved in the expression of penile erection and yawning induced by 5-HT1c receptor agonists.

Melis, M. R., R. Stancampiano, et al. (1995). "Nitroglycerin-induced penile erection and yawning in male rats: mechanism of action in the brain." *Brain Res Bull* 36(6): 527-31.

The effect of the central administration of nitroglycerin, a potent organic nitrate vasodilator, on penile erection and yawning was studied in male rats. When given intracerebroventricularly (ICV), nitroglycerin (33-99 micrograms) induced the above responses dose-dependently. The minimal effective dose was 33 micrograms, which was active in 60% of the rats. Nitroglycerin (1.65-6.6 micrograms) induced penile erection and yawning also when injected in the paraventricular nucleus of the hypothalamus. Nitroglycerin responses were prevented by methylene blue (200-400 micrograms ICV), by d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)<sub>2</sub>-Orn<sub>8</sub>-vasotocin (0.5-1 micrograms ICV) but not hemoglobin (100-200 micrograms ICV). In contrast methylene blue (10-20 micrograms), d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)<sub>2</sub>-Orn<sub>8</sub>-vasotocin (0.05-0.1 microgram) and hemoglobin (10-20 micrograms) were ineffective when injected in the paraventricular nucleus. Systemic haloperidol (0.5-1 mg/kg IP) was also ineffective. The results suggest that nitroglycerin induces penile erection and yawning by activating brain oxytocinergic transmission through the formation of nitric oxide in the paraventricular nucleus of the hypothalamus.

Melis, M. R., R. Stancampiano, et al. (1994). "Nitric oxide synthase inhibitors prevent N-methyl-D-aspartic acid-induced penile erection and yawning in male rats." *Neurosci Lett* 179(1-2): 9-12.

The effect of NG-nitro-L-arginine methylester (NAME) and N-mono-methyl-L-arginine (NMMA), inhibitors of nitric oxide (NO) synthase on penile erection and yawning induced by N-methyl-D-aspartic acid (NMDA) injected in the paraventricular nucleus of the hypothalamus (PVN) was studied in male rats. NAME (75-150 micrograms) and NMMA (250-500 micrograms), but not N-monomethyl-D-arginine (D-NMMA)(250-500 micrograms) prevented both responses in a dose-dependent manner when given intracerebroventricularly (i.c.v.) 15 min before NMDA (50 ng). NMDA-induced penile erection and yawning was also prevented by the guanylate cyclase inhibitor methylene blue (200-400 micrograms i.c.v.), but not by the NO scavenger methemoglobin (50-100 micrograms i.c.v.). NAME (10-20 micrograms), but not Methylene blue or methemoglobin (10-20 micrograms), prevented NMDA-induced responses also when injected in the PVN 15 min before NMDA. The present results suggest that NMDA-induced penile erection and yawning is mediated by an increased NO synthesis in the PVN.

Stancampiano, R., M. R. Melis, et al. (1994). "Penile erection and yawning induced by 5-HT1C receptor agonists in male rats: relationship with dopaminergic and oxytocinergic transmission." *Eur J Pharmacol* 261(1-2): 149-55.

1-(3-Chlorophenyl)piperazine (m-CPP) (0.1-4 mg/kg s.c.) and N-(3-trifluoromethylphenyl)-piperazine (TFMPP) (0.5-4 mg/kg s.c.), 5-HT1c receptor agonists, but not 8-hydroxy-dipropylamino-tetralin (8-OH-DPAT) (0.1 and 0.2 mg/kg s.c.), a 5-HT1A receptor agonist, induced penile erection and yawning with

a U-inverted dose-response curve in male rats. The maximal effect was found with 0.5 mg/kg s.c. of m-CPP and with 1 mg/kg s.c. of TFMPP. The m-CPP (0.5 mg/kg s.c.) and TFMPP (1 mg/kg s.c.) responses were prevented by mianserin (0.2 mg/kg s.c.) and by ritanserin (1 mg/kg s.c.) given 15 min before m-CPP and TFMPP. In contrast, m-CPP- or TFMPP-induced penile erection and yawning were not antagonized by haloperidol (0.1 mg/kg s.c.) or by [d(CH<sub>2</sub>)<sup>5</sup>Tyr(Me)<sub>2</sub>,Orn<sup>8</sup>]vasotocin (5 micrograms i.c.v.). Apomorphine- and oxytocin-induced penile erection, but not yawning, was also antagonized by mianserin and less effectively by ritanserin. The results suggest that 5-HT<sub>1C</sub> receptor agonist-induced penile erection and yawning are not mediated by increased dopaminergic and/or oxytocinergic transmission, and raise the possibility that a neuronal dopamine-oxytocin-5-HT link is involved in the control of penile erection and not necessarily of yawning in male rats.

Melis, M. R., R. Stancampiano, et al. (1994). "Prevention by NG-nitro-L-arginine methyl ester of apomorphine- and oxytocin-induced penile erection and yawning: site of action in the brain." *Pharmacol Biochem Behav* 48(3): 799-804.

The effect of NG-nitro-L-arginine methyl ester (NAME), a potent inhibitor of nitric oxide (NO) synthase, injected into different brain areas on penile erection and yawning induced by apomorphine or oxytocin was studied in male rats. The compound was found to be able to prevent the above behavioral responses dose dependently when injected into the paraventricular nucleus of the hypothalamus (PVN), but not in the caudate nucleus, medial septum, preoptic area, and the CA1 field of the hippocampus. When injected in the PVN, 5 micrograms of NAME induced a 30% reduction of apomorphine and oxytocin responses, while 20 micrograms induced an almost complete reduction. The effect of NAME seems to be related to the inhibition of guanylate cyclase secondary to the prevention of NO formation, because a dose-dependent reduction of apomorphine and oxytocin responses was obtained also with the inhibitor of guanylate cyclase methylene blue injected intracerebroventricularly (100-400 micrograms ICV), but not into the PVN. The results provide further support for a neurotransmitter role of central NO in the control of penile erection and yawning.

Melis, M. R., R. Stancampiano, et al. (1994). "Penile erection and yawning induced by paraventricular NMDA injection in male rats are mediated by oxytocin." *Pharmacol Biochem Behav* 48(1): 203-7.

The effect of N-methyl-D-aspartic acid (NMDA), (+-)-alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), or (+-)-trans-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) (5-60 ng in 0.3 microliter of saline) microinjected in the paraventricular nucleus of the hypothalamus on penile erection and yawning was studied in male rats. NMDA induced both penile erection and yawning in a dose-dependent manner. AMPA and ACPD also induced penile erection but less potently than NMDA, but were ineffective in causing yawning. NMDA effect on penile erection and yawning was prevented by (+)-MK-801 (0.05-0.1 mg/kg IP, 10 min before NMDA), by the oxytocin antagonist d(CH<sub>2</sub>)<sup>5</sup>Tyr(Me)-Orn<sup>8</sup>-vasotocin (50-100 ng ICV 10 min before NMDA), but not by haloperidol (0.1-0.5 mg/kg IP 10 min before NMDA). The results suggest that NMDA induces penile erection and yawning by increasing oxytocinergic transmission by acting in the paraventricular nucleus of the hypothalamus.

Melis, M. R., A. Mauri, et al. (1994). "Apomorphine- and oxytocin-induced penile erection and yawning in intact and castrated male rats: effect of sexual steroids." *Neuroendocrinology* 59(4): 349-54.

The effect of apomorphine (80 micrograms/kg s.c.) and oxytocin (30 ng i.c.v.) on penile erection and yawning was studied in intact and castrated male rats. In castrated rats both apomorphine and oxytocin responses were abolished. In these animals, testosterone (100 micrograms/kg s.c. once a day for 3 days), restored penile erection while estradiol benzoate (10 micrograms/kg s.c. once a day for 3 days) restored yawning induced by both compounds. 5-Dihydrotestosterone (DHT) or progesterone (each at a dose of 100 micrograms/kg s.c. once a day for 3 days) were ineffective. Given together, estradiol benzoate and DHT partially restored apomorphine- and oxytocin-induced yawning and penile erection, whereas estradiol benzoate and progesterone restored only yawning. Estradiol benzoate-induced recovery of yawning was prevented by the antiestrogen tamoxifen (1 mg/kg s.c. once a day for 3 days). In intact rats, progesterone increased and estradiol benzoate decreased apomorphine- and oxytocin-induced yawning without modifying penile erection, although oxytocin-induced yawning was prevented much less by estradiol benzoate than that induced by apomorphine. Testosterone or DHT were ineffective on both responses. Estradiol benzoate inhibition of apomorphine- and oxytocin-induced yawning was prevented by tamoxifen, which per se failed to modify apomorphine and oxytocin responses, as well as by testosterone or progesterone. The present results suggest that apomorphine- and oxytocin-induced penile erection and yawning are endocrine-dependent and differentially modulated by sexual steroids, suggesting that the mechanisms controlling the two behaviors are different even though they are often associated.

Melis, M. R. and A. Argiolas (1993). "Nitric oxide synthase inhibitors prevent apomorphine- and oxytocin-induced penile erection and yawning in male rats." *Brain Res Bull* 32(1): 71-4.

The effect of NG-nitro-L-arginine methyl ester and NG-monomethyl-L-arginine, two inhibitors of nitric oxide synthase, on apomorphine- and oxytocin-induced penile erection and yawning, was studied in male rats after intravenous and intracerebroventricular administration. Both compounds prevented dose-dependently apomorphine and oxytocin responses, when given systemically (5-50 mg/kg) or centrally (30-500 micrograms per rat), but NG-nitro-L-arginine methyl ester was about 5 times more potent than NG-monomethyl-L-arginine. The D-isomer of NG-monomethyl-L-arginine, which does not inhibit nitric oxide synthase, was ineffective. The results suggest that central nitric oxide is involved in the expression of penile erection and yawning induced by apomorphine and oxytocin.

Melis, M. R., R. Stancampiano, et al. (1992). "Effect of excitatory amino acid receptor antagonists on apomorphine-, oxytocin- and ACTH-induced penile erection and yawning in male rats." *Eur J Pharmacol* 220(1): 43-8.

The effect of excitatory amino acid receptor antagonists, (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5, 10-imine hydrogen maleate ((+)-MK-801), (+/-)-3-(2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and (+/-)-2-amino-4-phosphonobutanoic acid (AP-4), on penile erection and yawning induced by subcutaneous apomorphine (80 micrograms/kg), intracerebroventricular (i.c.v.) oxytocin (30 ng) and adrenocorticotropin (ACTH)-(1-24) (10 micrograms) was studied in male rats. Intraperitoneal (0.1-0.4 mg/kg) and i.c.v. (10-50 micrograms) (+)-MK-801 prevented dose dependently the penile erection and yawning induced by the three drugs. The (+)-MK-801 effect coincided with the appearance of head weaving, body rolling, hyperlocomotion and ataxia. Haloperidol (0.5 mg/kg i.p.) antagonized the prevention by (+)-MK-801 of oxytocin responses. Penile erection but not yawning was also prevented by high,

but not low doses of CPP and CNQX, which impaired motor performance, AP-4 was ineffective at all doses tested. The above compounds were ineffective when injected into the paraventricular nucleus of the hypothalamus, the brain area where apomorphine and oxytocin act to induce penile erection and yawning. The results suggest that excitatory amino acid transmission is not involved in the expression of penile erection and yawning induced by the above compounds.

Melis, M. R., R. Stancampiano, et al. (1992). "Hippocampal oxytocin mediates apomorphine-induced penile erection and yawning." *Pharmacol Biochem Behav* 42(1): 61-6.

Repeated episodes of penile erection and yawning can be induced in male rats either by low doses of the dopaminergic agonist apomorphine or by oxytocin given systemically or into a lateral ventricle (ICV), respectively, or after microinjection of the two substances directly in the paraventricular nucleus (PVN) of the hypothalamus. These behavioral responses are prevented in a dose-dependent manner by the ICV administration of the potent oxytocin antagonist d(CH<sub>2</sub>)<sup>5</sup>Tyr(Me)-Orn<sup>8</sup>-vasotocin. In contrast, the PVN injection of d(CH<sub>2</sub>)<sup>5</sup>Tyr(Me)-Orn<sup>8</sup>-vasotocin (1-30 ng), while effective in preventing oxytocin effect, was unable to prevent apomorphine response. On the other hand, apomorphine-, but not oxytocin-induced penile erection and yawning was prevented by electrolytic lesion of the medial septum (MS). Such a lesion decreased oxytocin content by about 45% in the hippocampus. The above results suggest that the hypothalamic-hippocampal oxytocinergic pathway mediates apomorphine-induced penile erection and yawning and that oxytocin is involved at different levels in the CNS for the control of these behavioral responses.

Stancampiano, R., M. R. Melis, et al. (1992). "Apomorphine- and oxytocin-induced penile erection and yawning in male rats: effect of pertussis toxin." *Brain Res Bull* 28(2): 315-8.

The effect of the intracerebroventricular (ICV) administration of pertussis toxin on penile erection and yawning induced by apomorphine and oxytocin was studied in male rats. Pertussis toxin (2 micrograms ICV) prevented the above behavioral responses to apomorphine (80 micrograms/kg SC) and oxytocin (30 ng ICV) on day 3 and 4, but not on day 0 and 1 after treatment. Oxytocin and apomorphine responses were restored on day 6. Similar results were obtained by microinjecting pertussis toxin (0.5 microgram) in the paraventricular nucleus of the hypothalamus, the most sensitive brain area for the induction of penile erection and yawning by oxytocin and apomorphine. The results suggest that G proteins are involved in the expression of above responses to apomorphine and oxytocin.

Melis, M. R., R. Stancampiano, et al. (1992). "Prevention by morphine of apomorphine- and oxytocin-induced penile erection and yawning: site of action in the brain." *Neuropsychopharmacology* 6(1): 17-21.

The effect of morphine administered systemically or into the paraventricular nucleus of the hypothalamus (PVN) on penile erection and yawning induced either by oxytocin or by the dopaminergic agonist apomorphine was studied in male rats. Systemic morphine (0.5 to 5 mg/kg intraperitoneally [IP]) prevented in a dose-dependent manner penile erection and yawning induced by the intracerebroventricular injection (ICV) of oxytocin (30 ng) or by the subcutaneous (SC) administration of apomorphine (80 micrograms/kg). Morphine (0.1 to 5 micrograms), but not U-69,593 (5 micrograms), injected into the PVN 10 minutes before oxytocin or apomorphine, was found to be able to prevent penile erection and yawning induced by the unilateral PVN microinjection of oxytocin

(10 ng) or apomorphine (50 ng). The morphine-induced prevention of these behavioral responses was abolished by pretreatment with naloxone (3 mg/kg IP) 15 minutes before morphine. The present results suggest that morphine prevents apomorphine- and oxytocin-induced penile erection and yawning by inhibiting the activity of oxytocinergic neurons through mu-type receptors in this hypothalamic nucleus.

Argiolas, A., M. R. Melis, et al. (1990). "Omega-conotoxin prevents apomorphine- and oxytocin-induced penile erection and yawning in male rats." *Pharmacol Biochem Behav* 37(2): 253-7.

The effect of the intracerebroventricular (ICV) administration of omega-conotoxin GVIA on penile erection and yawning induced by oxytocin or by the dopaminergic agonist apomorphine was studied in male rats. The peptide toxin, 1-10 ng given ICV 5 min before oxytocin (30 ng ICV) or apomorphine (80 micrograms/kg SC), but not its carboxymethylated (CM) derivative, prevented the above behavioral responses in a dose-dependent manner. Similarly, omega-conotoxin (5 ng) unilaterally injected in the paraventricular nucleus of the hypothalamus (PVN) prevented penile erection and yawning induced by the microinjection of oxytocin (10 ng) or apomorphine (50 ng) in the PVN. omega-Conotoxin injected in the PVN, but not in the preoptic area, prevented also penile erection and yawning induced by systemic apomorphine (80 micrograms/kg SC). ICV omega-conotoxin was unable to prevent stereotypy induced by apomorphine (500 micrograms/kg SC). The present results provide further evidence that calcium plays a major role in the expression of penile erection and yawning and that apomorphine and oxytocin induce these behavioral responses by mobilizing calcium through omega-conotoxin-sensitive (N-type) calcium channels.

Argiolas, A., M. R. Melis, et al. (1990). "Role of calcium in the expression of ACTH-induced stretching, yawning and penile erection." *Brain Res Bull* 24(6): 853-6.

The effect of omega-conotoxin GVIA, a potent and selective inhibitor of N-type calcium channels and of the organic calcium channel inhibitors nimodipine, verapamil and flunarizine, on stretching, yawning and penile erection induced by ACTH 1-24 was studied in male rats. omega-Conotoxin (1-10 ng ICV 15 min before ACTH, 10 micrograms ICV), but not carboxymethylated omega-conotoxin, induced a dose-dependent prevention of all ACTH effects. In contrast, organic calcium channel inhibitors (20 mg/kg IP 30-60 min before ACTH) failed to modify ACTH-induced stretching and yawning but induced a 25% decrease in the number of penile erection episodes induced by the peptide, and prevented, like ICV omega-conotoxin, oxytocin- and apomorphine-induced yawning and penile erection. When injected in the paraventricular nucleus of the hypothalamus, omega-conotoxin prevented the above behavioral responses induced by apomorphine and oxytocin but not by ACTH 1-24. The present results suggest that ACTH induces stretching, yawning and penile erection by mobilizing calcium through central omega-conotoxin-sensitive calcium channels in brain sites different from those sensitive to oxytocin and apomorphine.

Argiolas, A., M. R. Melis, et al. (1990). "Oxytocin-induced penile erection and yawning: role of calcium and prostaglandins." *Pharmacol Biochem Behav* 35(3): 601-5.

The effect of verapamil, flunarizine, nimodipine, nifedipine, calcium channel inhibitors, and of indomethacin and aspirin, inhibitors of prostaglandin synthesis, on penile erection and yawning induced by oxytocin was studied in male rats. All calcium channel inhibitors given

intraperitoneally (IP) 60 min before the intracerebroventricular (ICV) injection of oxytocin (30 ng) prevented in a dose-dependent manner oxytocin effect. Nimodipine and nicardipine were the most effective being active at doses between 5 and 20 mg/kg, while the others were active at doses higher than 15 mg/kg. Prevention of oxytocin effect was also seen after ICV injection of the above compounds. Unlike calcium channel inhibitors, indomethacin given either IP (10 and 50 mg/kg) or ICV (50 micrograms), or aspirin (100 mg/kg IP) were ineffective. Microinjection of calcium, but not of prostaglandin E2 and prostaglandin F2 alpha in the paraventricular nucleus of the hypothalamus, the brain area most sensitive for the induction of the above behavioral responses by oxytocin, induced a symptomatology similar to that induced by oxytocin. The present results suggest that calcium might be the second messenger which mediates the expression of penile erection and yawning induced by oxytocin.

Melis, M. R., A. Argiolas, et al. (1989). "Oxytocin-induced motor disturbances: relationship with penile erection and yawning." *Pharmacol Biochem Behav* 34(3): 673-5.

The intracerebroventricular (ICV) injection of oxytocin at doses between 5 and 100 ng induced repeated episodes of penile erection and yawning, while at doses between 100 ng and 10 micrograms it induced motor disturbances often culminating in barrel rotation in rats. The intensity of motor disturbances was inversely correlated to the number of yawning and penile erection episodes. Pretreatment with the dopaminergic agonist apomorphine (80 and 240 micrograms/kg SC) failed to modify the incidence of motor disturbance induced by high doses of oxytocin, but markedly reduced the intensity of the symptomatology in a dose-dependent manner. The present results suggest that high doses of oxytocin induce motor disturbances which mask penile erection and yawning, and that brain dopaminergic systems have a protective role against this symptomatology.

Argiolas, A., M. R. Melis, et al. (1989). "Calcium channel inhibitors prevent apomorphine- and oxytocin-induced penile erection and yawning in male rats." *Eur J Pharmacol* 166(3): 515-8.

The effect of i.p. injection of the calcium channel inhibitors, verapamil, flunarizine, nifedipine, nimodipine and nicardipine, on penile erection and yawning induced by the dopamine agonist, apomorphine, or by oxytocin was studied in male rats. The five compounds antagonized in a dose-dependent manner the behavioral responses induced either by apomorphine or oxytocin. Nimodipine and nicardipine were found to be the most potent, being active in doses between 5 and 20 mg/kg, while nifedipine, verapamil and flunarizine were active in doses higher than 15 mg/kg. The results suggest that calcium is involved in the expression of the above-mentioned behavioural responses.

Melis, M. R., A. Argiolas, et al. (1989). "Evidence that apomorphine induces penile erection and yawning by releasing oxytocin in the central nervous system." *Eur J Pharmacol* 164(3): 565-70.

Oxytocin (10 and 30 ng) injected into a lateral ventricle (i.c.v.) or the dopamine agonist apomorphine (40 and 80 micrograms/kg) injected subcutaneously induced repeated episodes of penile erection and yawning in male rats. The concomitant administration of the two substances did not produce any further increase in the number of penile erection and yawning episodes. Penile erection and yawning induced by either oxytocin or apomorphine were antagonized in a dose-dependent manner by i.c.v. pretreatment with the oxytocin antagonists [d(CH<sub>2</sub>)<sup>5</sup>Tyr(Me)-Orn<sup>8</sup>]vasotocin, [Pen<sup>1</sup>,Phe(Me)<sup>2</sup>,Thr<sup>4</sup>,Orn<sup>8</sup>]oxytocin and [d(CH<sub>2</sub>)<sup>5</sup>Tyr(Me)-Arg<sup>8</sup>]vasopressin, with a rank order of potency that follows

their antioxytotic activity. (i.e. [d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)-Orn<sub>8</sub>]vasotocin congruent to [Pen<sub>1</sub>,Phe(Me)<sub>2</sub>,Thr<sub>4</sub>,Orn<sub>8</sub>]-oxytocin greater than [d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)-Arg<sub>8</sub>]vasopressin). The results suggest that apomorphine induces penile erection and yawning by releasing oxytocin in the central nervous system.

Argiolas, A., M. R. Melis, et al. (1989). "Penile erection and yawning induced by oxytocin and related peptides: structure-activity relationship." *Peptides* 10(3): 559-63.

The potency of several oxytocin-related peptides in inducing penile erection and yawning after injection into a lateral ventricle of male rats was compared. Substitution of two amino acids in the oxytocin molecule or deletion of the C-terminal glycinamide as in des-GlyNH<sub>2</sub>-oxytocin [oxytocin(1-8)] reduced oxytocin potency in inducing both effects, the rank order being: oxytocin greater than [Thr<sub>4</sub>,Gly<sub>7</sub>]-oxytocin congruent to isotocin [( Ser<sub>4</sub>,Ile<sub>8</sub>)-oxytocin] greater than vasopressin [( Phe<sub>3</sub>,Arg<sub>8</sub>)-oxytocin] greater than des-GlyNH<sub>2</sub>-oxytocin. Oxytocin's ability to induce penile erection and yawning was abolished by permanent opening of the disulfide bridge by reduction and carboxymethylation. Oxytocin(1-6) and oxytocin(7-9) were also inactive. Penile erection and yawning induced by oxytocin-related peptides were antagonized in a dose-dependent manner by nonapeptide antagonists with a rank order of potency that follows their antioxytotic activity (d[(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)Orn<sub>8</sub>]-vasotocin congruent to [Pen<sub>1</sub>,Phe(Me)<sub>2</sub>,Thr<sub>4</sub>,Orn<sub>8</sub>]-oxytocin greater than d[(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)Arg<sub>8</sub>]-vasopressin). Carboxymethylated oxytocin, oxytocin(1-6), and oxytocin(7-9) were devoid of antagonistic activity. The present results suggest that central oxytocin receptors mediating the expression of penile erection and yawning are structurally related to those present in the uterus and in the mammary gland.

Argiolas, A., M. R. Melis, et al. (1989). "Oxytocin-induced penile erection and yawning in male rats: effect of neonatal monosodium glutamate and hypophysectomy." *Psychopharmacology (Berl)* 97(3): 383-7.

Penile erection and yawning induced by the intracerebroventricular (ICV) injection of oxytocin (10-1000 ng) was studied in hypophysectomized rats and in rats neonatally treated with monosodium glutamate (MSG), a treatment that depletes hypothalamic opiomelanocorticotropin-derived peptides without altering their pituitary and circulating concentration. Oxytocin effect was strongly reduced by hypophysectomy, but not by neonatal MSG. Testosterone replacement (50 micrograms/kg/day for 23 days) partially reversed the effect of hypophysectomy on penile erection, but not on yawning. The present results suggest that oxytocin does not induce penile erection and yawning by releasing an ACTH-derived peptide from hypothalamic opiomelanotropinergic neurons, and that the pituitary gland exerts a permissive role on the expression of the above behavioural responses induced by oxytocin.

Melis, M. R., A. Argiolas, et al. (1988). "Oxytocin-induced penile erection and yawning: structure-activity relationship studies." *Pharmacol Res Commun* 20(12): 1117-8.

Argiolas, A., M. R. Melis, et al. (1988). "Central dopamine-oxytocin-adrenocorticotropin link in the expression of yawning and penile erection." *Ann Ist Super Sanita* 24(3): 377-82.

Argiolas, A., M. R. Melis, et al. (1988). "Yawning and penile erection: central dopamine-oxytocin-adrenocorticotropin connection." *Ann N Y Acad Sci* 525: 330-7.

Argiolas, A., M. R. Melis, et al. (1987). "Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats." *Brain Res* 421(1-2): 349-52.

The effect of electrolytic lesion of the paraventricular nucleus of the hypothalamus (PVN) on yawning and penile erection induced by apomorphine, oxytocin and adrenocorticotrophic hormone (ACTH1-24) was studied in male rats. In sham-operated rats, apomorphine (50 micrograms/kg s.c.), oxytocin (30 ng i.c.v.), and ACTH1-24 (10 micrograms i.c.v.) significantly increased the number of yawning and penile erection episodes. In PVN-lesioned rats, apomorphine- and oxytocin-, but not ACTH-induced responses were strongly reduced. These results confirm our previous observations showing that the PVN has a crucial role in the expression of yawning and penile erection induced by dopamino-mimetic drugs and oxytocin, and suggest that ACTH-derived peptides induce the above responses by a mechanism not involving PVN hypothalamic dopamine or oxytocin.

Melis, M. R., A. Argiolas, et al. (1987). "Apomorphine-induced penile erection and yawning: site of action in brain." *Brain Res* 415(1): 98-104.

Microinjection of the dopamine (DA) agonist apomorphine into the paraventricular nucleus of the hypothalamus (PVN) induced penile erection and yawning in rats. A significant effect was elicited by a dose of apomorphine as low as 5 ng. The symptomatology usually began within 5 min after the microinjection, lasted for 30-50 min, and was identical to that induced by the systemic administration of the drug. Stereotypy and hypermotility were never observed after apomorphine microinjection into the PVN, even at the highest dose tested (1 microgram). Microinjections of the same doses of apomorphine into the hypothalamic ventromedial and dorsomedial nucleus, preoptic area, caudate nucleus, nucleus accumbens and substantia nigra, were ineffective. LY 171555, a specific D2 Da receptor agonist, and (+)-3-PPP, but not (-)-3-PPP nor the specific D1 DA receptor agonist SKF 38393, were as effective as apomorphine when injected into the PVN. Apomorphine-induced penile erection and yawning were antagonized by pretreatment with neuroleptic drugs, such as haloperidol, (-)-sulpiride, a specific D2 DA antagonist, and SCH 23390, a specific D1 DA antagonist. The present results suggest that the PVN is the brain area where D2 DA agonists act to induce penile erection and yawning. Moreover, since the PVN contains the cell bodies of a group of incerto-hypothalamic DA neurons, the above results suggest for the first time a possible involvement of the incerto-hypothalamic DA system in the expression of penile erection and yawning.

Argiolas, A., M. R. Melis, et al. (1987). "Monosodium glutamate does not alter ACTH- or apomorphine-induced penile erection and yawning." *Pharmacol Biochem Behav* 26(3): 503-7.

The effect of the intracerebroventricular (ICV) injection of ACTH 1-24 (1, 5 and 10 micrograms) or the subcutaneous administration of apomorphine (20 and 80 micrograms/kg SC) on spontaneous penile erection and yawning was studied in rats treated with monosodium glutamate (MSG), a treatment that depletes hypothalamic ACTH, alpha-MSH and endorphin-like peptides. Neonatal MSG treatment failed to antagonize either apomorphine- or ACTH-induced yawning in male and female rats, or to alter the number of penile erection episodes induced by the two substances in male rats. In contrast, hypophysectomy, that does not alter the concentration of hypothalamic ACTH and alpha-MSH, caused a marked prevention of apomorphine- and ACTH-induced responses, in agreement with previous studies. The results suggest that the integrity of opiomelanotropinergic neurons in the hypothalamus is not necessary for the induction of yawning and penile erection

by ACTH-derived peptides, and that apomorphine and other dopamine agonists apparently do not induce penile erection and yawning by releasing an ACTH-derived peptide in brain.

Argiolas, A., M. R. Melis, et al. (1987). "d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)-[Orn<sub>8</sub>]vasotocin, a potent oxytocin antagonist, antagonizes penile erection and yawning induced by oxytocin and apomorphine, but not by ACTH-(1-24)." *Eur J Pharmacol* 134(2): 221-4.

Intraventricular (i.c.v.) injection of d(CH<sub>2</sub>)<sub>5</sub>-Tyr(Me)-[Orn<sub>8</sub>]vasotocin, a potent oxytocin antagonist, antagonized in a dose-dependent manner (10-100 ng) penile erection and yawning induced by the systemic injection of apomorphine (80 micrograms/kg s.c.) or by the i.c.v. injection of oxytocin (30 ng). In contrast, the oxytocin antagonist, even at the dose of 10 micrograms, did not modify penile erection and yawning induced by the i.c.v. injection of ACTH-(1-24). These results suggest that apomorphine, but not ACTH-(1-24), induce penile erection and yawning by releasing oxytocin in some brain area.

Argiolas, A., M. R. Melis, et al. (1987). "Yawning: neurochemistry, physiology and pathology." *Cephalalgia* 7 Suppl 6: 131-7.

Melis, M. R., A. Argiolas, et al. (1986). "Oxytocin-induced penile erection and yawning: site of action in the brain." *Brain Res* 398(2): 259-65.

Microinjection of oxytocin into the paraventricular nucleus of the hypothalamus or into the CA1 field of the hippocampus induced a dose-dependent increase in the number of penile erection and yawning episodes in male rats. The minimal effective dose of oxytocin injected into the paraventricular nucleus was 3 ng. This dose induced the above-mentioned behaviors in 60% of the treated rats. Doses of 9 ng or higher induced the symptomatology in more than 85% of the animals. On the other hand, when the peptide was injected into the CA1 field of the hippocampus, 9 ng bilaterally were necessary to elicit penile erection and yawning in 62% of the rats. Arg<sub>8</sub>-vasopressin, which only differs from oxytocin in two amino acids, induced penile erection and yawning when injected either into the paraventricular nucleus or into the hippocampus, but was 5-10 times less potent than oxytocin. Oxytocin injection into the lateral septum, caudate nucleus, subiculum, preoptic area, ventromedial nucleus and supraoptic nucleus, was ineffective. The powerful effect of oxytocin on the induction of yawning and penile erection, suggests a physiological role of hypothalamic and hippocampal oxytocin in the regulation of such responses.

Argiolas, A., M. R. Melis, et al. (1986). "Oxytocin: an extremely potent inducer of penile erection and yawning in male rats." *Eur J Pharmacol* 130(3): 265-72.

The intracerebroventricular (i.c.v.) injection of oxytocin, in doses ranging from 5 to 90 ng (5-90 pmol) induced penile erection and yawning in male rats. Such response was not induced by doses of the peptide higher than 100 ng, nor by equimolar doses of i.c.v. [Arg<sub>8</sub>]vasopressin, ACTH-(1-24), alpha-MSH, rat corticotropin-releasing factor (rCRF), delta sleep-inducing peptide, neurotensin or substance P. Oxytocin-induced penile erection and yawning were prevented by atropine and morphine, but not by methylatropine or the opiate antagonist naloxone. Haloperidol, a dopamine receptor antagonist, was ineffective at low doses; it partially prevented penile erection but not yawning at high doses. Since oxytocin is present not only in the neurohypophysis but also in other brain areas, our results suggest that oxytocin is implicated in the regulation of penile erection and yawning, and provide further evidence that oxytocin acts as a neuropeptide in the central nervous system.

Argiolas, A., M. R. Melis, et al. (1985). "Intraventricular oxytocin induces yawning and penile erection in rats." *Eur J Pharmacol* 117(3): 395-6.

Argiolas, A. and M. R. Melis (19980205). "The neuropharmacology of yawning." *European Journal of Pharmacology* 343(1): 1-16.

Yawning is a phylogenetically old, stereotyped event that occurs alone or associated with stretching and/or penile erection in humans and in animals from reptiles to birds and mammals under different conditions. Although its physiological function is still unknown, yawning is under the control of several neurotransmitters and neuropeptides at the central level as this short overview of the literature on the neurochemistry of yawning shows. Among these substances, the best known are dopamine, excitatory amino acids, acetylcholine, serotonin, nitric oxide, adrenocorticotrophic hormone-related peptides and oxytocin, that facilitate yawning and opioid peptides that inhibit this behavioral response. Some of the above compounds interact in the paraventricular nucleus of the hypothalamus to control yawning. This hypothalamic nucleus contains the cell bodies of oxytocinergic neurons projecting to extra-hypothalamic brain areas that play a key role in the expression of this behavioral event. When activated by dopamine, excitatory amino acids and oxytocin itself, these neurons facilitate yawning by releasing oxytocin at sites distant from the paraventricular nucleus, i.e. the hippocampus, the pons and/or the medulla oblongata. Conversely, activation of these neurons by dopamine, oxytocin or excitatory amino acids, is antagonized by opioid peptides, that, in turn, prevent the yawning response. The activation and inhibition, respectively of these oxytocinergic neurons is related to a concomitant increase and decrease, respectively, of paraventricular nitric oxide synthase activity. However, other neuronal systems in addition to the central paraventricular oxytocinergic neurons are involved in the control of yawning, since they do not seem to be involved in the expression of yawning induced by the stimulation of acetylcholine or serotoninergic receptors, nor by adrenocorticotrophic hormone (ACTH) and related peptides. Nitric oxide is also involved in the induction of yawning by the latter compounds and neuronal links, for instance between dopamine and acetylcholine and dopamine and serotonin, seem to be involved in the yawning response. Finally, other neurotransmitters, i.e.  $\gamma$ -aminobutyric acid (GABA) and noradrenaline, and neuropeptides, i.e. neurotensin and luteinizing hormone-releasing hormone (LH-RH), influence this behavioral response. In conclusion, in spite of some recent progress, little is known of, and more has to be done to identify, the neurochemical mechanisms underlying yawning at the central level.

Argiolas, A., M. R. Melis, et al. (200004). "ACTH- and  $\alpha$ -MSH-induced grooming, stretching, yawning and penile erection in male rats: Site of action in the brain and role of melanocortin receptors." *Brain Research Bulletin* 51(5): 425-431.

The effect of adrenocorticotropin (ACTH)(1-24) and  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) on grooming, stretching, yawning and penile erection was studied after injection into different brain areas. Both peptides induce the above responses when injected into the hypothalamic periventricular region of the third ventricle. This region includes the paraventricular nucleus, the dorsomedial nucleus, the ventromedial nucleus and the anterior hypothalamic area. The minimal effective dose of both peptides was 0.5  $\mu$ g and the maximal effect was seen with 2  $\mu$ g, the highest dose tested. Irrespective of the injection site, grooming started 5 $\pm$ 7 min after injection of either peptide, while stretching, yawning and penile erection started only after 15 $\pm$ 35 min and

lasted for 90±120 min. In contrast both peptides were ineffective when injected into the preoptic area, the caudate nucleus or the CA1 field of the hippocampus. Grooming, stretching and yawning, but not penile erection, were prevented by cyclic[AcCys11, D-Nal14, Cys18, AspNH222]-fl-MSH (11-22) (HS014), a selective melanocortin 4 receptor antagonist, injected into the same periventricular area 10 min before of ACTH(1-24) or  $\alpha$ -MSH. The results show that ACTH(1-24) and  $\alpha$ -MSH act in the hypothalamic periventricular region to induce the above responses and that grooming, stretching and yawning, but not penile erection, are mediated by melanocortin 4 receptors.

Rosaria Melis, M., M. S. Spano, et al. (20000310). "Activation of  $\gamma$ -aminobutyric acidA receptors in the paraventricular nucleus of the hypothalamus reduces apomorphine-, N-methyl-d-aspartic acid- and oxytocin-induced penile erection and yawning in male rats." *Neuroscience Letters* 281(2-3): 127-130.

The effect of muscimol and baclofen injected into the paraventricular nucleus of the hypothalamus on penile erection and yawning induced by apomorphine, oxytocin and N-methyl-d-aspartic acid (NMDA) was studied in male rats. Muscimol (20±200 ng), but not baclofen (200 ng), injected into the paraventricular nucleus of the hypothalamus 10 min before apomorphine (50 ng), oxytocin (10 ng) or NMDA (50 ng) reduced penile erection and yawning induced by the above compounds given into the paraventricular nucleus. Bicuculline (250 ng) injected into the paraventricular nucleus 5 min before muscimol (100 ng) prevented the inhibitory effect of muscimol on penile erection and yawning induced by apomorphine, oxytocin and NMDA. The present results show that  $\gamma$ -aminobutyric acid (GABA) inhibits penile erection and yawning by acting on GABAA receptors in the paraventricular nucleus of the hypothalamus.

Succu, S., M. S. Spano, et al. (19981016). "Different effects of  $\gamma$ -conotoxin on penile erection, yawning and paraventricular nitric oxide in male rats." *European Journal of Pharmacology* 359(1): 19-26.

A dose of apomorphine or oxytocin that induces penile erection and yawning increases nitric oxide production in the paraventricular nucleus of the hypothalamus, as determined by the increase in NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> concentration induced by these substances in the paraventricular dialysate obtained from male rats. All the above responses were prevented by a dose of  $\gamma$ -conotoxin-GVIA as low as 5 ng. This potent inhibitor of N-type Ca<sup>2+</sup> channels was injected into the paraventricular nucleus 15 min before apomorphine (50 ng) or oxytocin (10 ng). In contrast,  $\gamma$ -conotoxin was ineffective when the above responses were induced by N-methyl-d-aspartic acid (50 ng). The peptide toxin (5 ng) was also ineffective on the penile erection and yawning induced by the nitric oxide donors sodium nitroprusside (50  $\mu$ g) or hydroxylamine (50  $\mu$ g), injected into the paraventricular nucleus. The present results suggest that  $\gamma$ -conotoxin-sensitive Ca<sup>2+</sup> channels are involved in the activation of nitric oxide synthase, penile erection and yawning induced by apomorphine and oxytocin, but not by N-methyl-d-aspartic acid, at the paraventricular level.

Melis, M. R., S. Succu, et al. (19970326). "Oxytocin increases nitric oxide production in the paraventricular nucleus of the hypothalamus of male rats: correlation with penile erection and yawning." *Regulatory Peptides* 69(2): 105-111.

A dose of oxytocin (50 ng i.c.v.) that induces penile erection and yawning, increased the concentration of NO<sub>2</sub><sup>-</sup> from 0.98±0.29 to 4.2±0.79  $\mu$ M and of NO<sub>3</sub><sup>-</sup> from 5.6±0.33 to 12.03±0.99  $\mu$ M in the dialysate from the paraventricular nucleus of the hypothalamus of male rats, as measured by in vivo microdialysis.

NO<sub>2</sub><sup>-</sup> concentration was also increased by [Thr<sup>4</sup>,Gly<sup>7</sup>]-oxytocin (100 ng i.c.v.) and oxytocin(1-8) (1 µg i.c.v.), which also induced penile erection and yawning, but not by oxytocin(1-6) (1 µg i.c.v.) or oxytocin(7-9) (1 µg i.c.v.), which were unable to induce these behavioral responses. The oxytocin effect on NO<sub>2</sub><sup>-</sup> concentration, penile erection and yawning was prevented by the oxytocin receptor antagonist, d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)-Orn<sup>8</sup>-vasotocin (1 µg i.c.v.), or by the nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (200 µg i.c.v.), but not by the dopamine receptor antagonist, haloperidol (0.5 mg/kg i.p.). The nitric oxide scavenger, hemoglobin (200 µg i.c.v.), prevented oxytocin-induced NO<sub>2</sub><sup>-</sup> concentration increase, but was unable to prevent penile erection and yawning. Methylene blue (300 µg i.c.v.), an inhibitor of guanylate cyclase, was ineffective on oxytocin-induced NO<sub>2</sub><sup>-</sup> concentration increase, but prevented the behavioral responses. The results suggest that oxytocin induces penile erection and yawning by increasing nitric oxide synthase activity in the cell bodies of oxytocinergic neurons projecting to extra-hypothalamic brain areas and mediating the behavioral responses.

Melis, M. R., S. Succu, et al. (1997). "N-Methyl-d-aspartic acid-induced penile erection and yawning: role of hypothalamic paraventricular nitric oxide." *European Journal of Pharmacology* 328(2-3): 115-123.

A dose of N-methyl-d-aspartic acid (NMDA, 50 ng) that induces penile erection and yawning when injected into the paraventricular nucleus of the hypothalamus, increased the concentration of NO<sub>2</sub><sup>-</sup> from 1.10±0.28 µM to 7.32±1.12 µM and of NO<sub>3</sub><sup>-</sup> from 4.96±0.69 µM to 10.5±1.61 µM in the paraventricular dialysate obtained from male rats by *in vivo* microdialysis. NO<sub>2</sub><sup>-</sup> concentration was not increased by (±)-α-(amino)-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 100 ng) or by trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) (100 ng), which were unable to induce these behavioral responses. N-Methyl-d-aspartic acid effect on NO<sub>2</sub><sup>-</sup> concentration, penile erection and yawning was prevented by dizolcipine (MK-801) (10-100 ng) or by the nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester (20 µg), but not by the oxytocin receptor antagonist [d(CH<sub>2</sub>)<sub>5</sub>,Tyr(Me)<sub>2</sub>,Orn<sup>8</sup>]vasotocin (100 ng), or by the guanylate cyclase inhibitor methylene blue (20 µg) given in the paraventricular nucleus 15 min before N-methyl-d-aspartic acid or by the dopamine receptor antagonist haloperidol (0.5 mg/kg) given intraperitoneally 30 min before N-methyl-d-aspartic acid. In contrast, the nitric oxide scavenger hemoglobin (20 µg) given in the paraventricular nucleus prevented N-methyl-d-aspartic acid-induced NO<sub>2</sub><sup>-</sup> concentration increase, but was unable to prevent penile erection and yawning. The results suggest that N-methyl-d-aspartic acid induces penile erection and yawning by increasing nitric oxide synthase activity in the paraventricular nucleus of the hypothalamus, possibly in the cell bodies of oxytocinergic neurons projecting to extra-hypothalamic brain areas and mediating these behavioral responses.

Melis, M. R. and A. Argiolas (2002). "Reduction of drug-induced yawning and penile erection and of noncontact erections in male rats by the activation of GABAA receptors in the paraventricular nucleus: involvement of nitric oxide." *Eur J Neurosci* 15(5): 852-60.

The effect of muscimol, a GABAA receptor agonist, injected into the paraventricular nucleus (PVN) of the hypothalamus on drug-induced (apomorphine, oxytocin and NMDA) yawning and penile erection, and on the increase in the concentration of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> occurring in the paraventricular dialysate in these experimental conditions, was studied in male rats. Muscimol (50, 100 and 200 ng) reduced, in a dose-dependent manner, penile erection and yawning induced

by apomorphine (50 ng), oxytocin (30 ng) and NMDA (50 ng) delivered into the PVN. The reduction of penile erection and yawning was parallel to a reduction of the concomitant NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> increase that occurs in the paraventricular dialysate in this experimental condition. In contrast, baclofen (200 ng), a GABAB receptor agonist, was ineffective. The muscimol effects on drug-induced penile erection, yawning and NO<sub>2</sub><sup>-</sup> increase were prevented by the prior administration of bicuculline (250 ng into the paraventricular nucleus). Muscimol (200 ng) but not baclofen (200 ng), injected into the PVN, reduced both noncontact erections in male rats placed in the presence of an inaccessible receptive female, and also the NO<sub>2</sub><sup>-</sup> increase that occurs in the paraventricular dialysate in this experimental condition. As found with drug-induced penile erection, the muscimol reduction of noncontact erections and of NO<sub>2</sub><sup>-</sup> increase was prevented by bicuculline. The present results show that the activation of GABAA receptors in the PVN reduces yawning and penile erection induced by drugs or physiological stimuli by reducing the increase in NO activity that occurs in this hypothalamic nucleus in these experimental conditions.