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Review

The neuropharmacology of yawning

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Abstract

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, adrenocorticotropic 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 form the paraventricular nucleus, i.e. the hippocampus, the pons and/or the medulla oblongata. Conversely, activation of these neurons by dopamine, oxytocin or excitatory aminoacids, 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 adrenocorticotropic 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. γ-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.

Keywords: Yawning; Dopamine; Acetylcholine; 5-HT (5-hydroxytryptamine, serotonin); Excitatory amino acid; ACTH–MSH peptide; Oxytocin; Opioid peptide; Nitric oxide (NO); GABA; Noradrenaline; Neurotensin; Luteinizing hormone–releasing hormone (LH-RH); Paraventricular nucleus of the hypothalamus; (Rat)

1. 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 (see Vischer, 1959; Lehmann, 1979). It is characterized by gaping accompanied by a long inspiration, followed by a shorter expiration and resembles classical reflexes, because once initiated, the specific pattern of motor output with associated inspiration/expiration (yawn) goes to completion with minimal influence of a sensory feedback. However yawning is not a simple reflex of short duration, but has a complex spatio-temporal organization with facial, respiratory and other components. Since it is also seen in anencephalic newborns with only the medulla oblongata (see Price Heusner, 1946), the neural structures necessary for yawning are presumably located in the brainstem near or within other respiratory and vasomotor centers, especially those that control facial mimics, mastication, throat and respiration and possibly stretching (see below). The

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internal physiological stimuli that evoke spontaneous yawning as well as 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 (see Provine, 1986; Provine et al., 1987a,b and references therein). 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 (Provine, 1986; Provine et al., 1987a,b and references therein). Its more frequent occurrence at bed and waking times and in boring situations than at other times of the day and situations, together 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 (see Bertolini and Gessa, 1981 and references therein). This hypothesis is, however, far from being generally accepted (see Provine, 1986; Provine et al., 1987a,b). Yawning can be also observed in other contexts, for instance before eating (see Holmgren et al., 1991 and references therein), in the presence of nausea, motion sickness, brain tumors or lesions, hemorrhage and encephalitis (Price Heusner, 1946; Barbizet, 1958; Jurko and Andy, 1975; Lehmann, 1979 and references therein), or after several neuropharmacological manipulations (see below). The present aim was to survey briefly the neuropharmacological studies in particular, because they have revealed that the occurrence of yawning alone, but more often associated with stretching and penile erection (see Ferrari et al., 1963; Bertolini and Gessa, 1981; Holmgren et al., 1985; Argiolas et al., 1986; Urba-Holmgren et al., 1990), is under the control of several neurotransmitters and neuropeptides at the central level. Of these, the best known are adrenocorticotropin (ACTH), α-melanocyte stimulating hormone (α-MSH) and related peptides, acetylcholine, dopamine, serotonin (5-HT), excitatory amino acids, nitric oxide (NO), oxytocin and opioid peptides (Tables 1–3).

2. ACTH–MSH peptides

The studies suggesting that neuropeptides and/or neurotransmitters are involved in the control of yawning, started in the sixties with the discovery that ACTH, α-MSH and related peptides induced the so called 'stretching–yawning syndrome' in several laboratory animals (dogs, cats, rabbits, rats, mice and so on) when injected in the central nervous system but not peripherally (Ferrari et al., 1963; Gispen et al., 1975; Bertolini and Gessa, 1981 and references therein). The stretching–yawning syndrome induced by ACTH–MSH-related peptides is peculiar in several aspects. First, it starts only 25–30 min after the treatment and, second, it usually lasts for hours. The minimal active sequence inducing the symptomatology was found to be the hexapeptide ACTH-(4–9), that is also contained in the α-MSH molecule. The hypothalamus was identified as one of the brain areas where ACTH–MSH peptides apparently act to induce the stretching–yawning syndrome (Gessa et al., 1967). The ACTH-induced stretching–yawning syndrome was abolished by hypophysectomy but not by neonatal monosodium glutamate (see Argiolas et al., 1987a), a treatment that it is now known to deplete ACTH–MSH peptides in the central nervous system (see below), nor by castration, that eliminates ACTH-induced penile erection and ejaculation which occur concomitantly with stretching and yawning (see Bertolini and Gessa, 1981).

In the seventies, it was still unknown that ACTH and α-MSH, together with β-endorphin, are derived from pro-opiomelanocortin and that pro-opiomelanocortin-containing neurons are present in the brain (see O'Donohue and Dorsa, 1982 and references therein). Nevertheless, even after the discovery of these pro-opiomelanocortin-containing neurons, which suggested the existence of ACTH and α-MSH receptors in the central nervous system, it proved very difficult to find specific receptors for these peptides in the central nervous system, despite the clear demonstration of ACTH receptors linked to adenylate cyclase in the adrenal gland (Ontjes et al., 1977). This has so far ham-

Table 1
Effects of neurotransmitters that influence yawning by acting at central level

Neurotransmitter	Effect on yawning	Receptor involved	Brain area involved	Neuronal systems involved
Dopamine	facilitatory	D_2	PVN, CN, Se	incertohypothalamic, nigrostriatal
Serotonin	facilitatory	5-HT_{2C}	not available	not available
	inhibitory	5-HT_{1A}	not available	not available
	inhibitory	5-HT_2	not available	not available
Acetylcholine	facilitatory	M_1, M_2	HI, others	septohippocampal
Excitatory amino acids	facilitatory	NMDA	PVN	not available
Nitric oxide	facilitatory	n.a.	PVN	oxytocinergic
GABA	inhibitory	GABA-B	not available	not available
Noradrenaline	facilitatory	α	not available	not available
	inhibitory	β	not available	not available

See text for references. PVN = paraventricular nucleus of the hypothalamus; HI = hippocampus, CN = caudate nucleus, Se = septum.

Table 2
Amino acid sequence of neuropeptides that influence yawning by acting in the central nervous system

ACTH	Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–Ala–Glu–Asp–Glu–Ser–Ala–Glu–Ala–Phe–Pro–Leu–Glu–Phe
α-MSH	AcSer–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–$ValNH_2$
Met-E	Tyr–Gly–Gly–Phe–Met
Leu-E	Tyr–Gly–Gly–Phe–Leu
β-END	Tyr–Gly–Gly–Phe–Met–Thr–Ser–Glu–Lys–Ser–Gln–Thr–Pro–Leu–Val–Thr–Leu–Phe–Lys–Asn–Ala–Ile–Ile–Lys–Asn–Ala–Tyr–Lys–Lys–Gly–Gln
Dynorfin	Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg–Pro–Lys–Leu–Lys–Trp–Asp–Asn–Gln
Oxytocin	Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–$GlyNH_2$
Neurotensin	pGlu–Leu–Tyr–Glu–Asn–Lys–Pro–Arg–Arg–Pro–Tyr–Ile–Leu
LH-RH	pGlu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–$GlyNH_2$

ACTH = adrenocorticotropic hormone; α-MSH = α-melanocyte stimulating hormone; Met- and Leu-E = methionine- and leucine-enkephalin; β-END = β-endorphin; LH-RH = luteinizing hormone-releasing hormone.

Table 3
Effects of neuropeptides that influence yawning by acting at central level

Neuropeptides	Effect on yawning	Receptor involved	Brain area involved
ACTH/MSH peptides	facilitatory	n.a.	HY
Opioid peptides	inhibitory	μ	PVN, others
Oxytocin	facilitatory	uterine-type	PVN, HI, MO, pons
Neurotensin	inhibitory	n.a.	n.a.
LH-RH	inhibitory	n.a.	n.a.
Prolactin	facilitatory	n.a.	CN

Appropriate references are quoted in the text. ACTH–MSH = adrenocorticotropin–melanocyte-stimulating hormone; LH-RH = luteinizing hormone-releasing hormone. PVN = paraventricular nucleus of the hypothalamus, HI = hippocampus, HY = hypothalamus, CN = caudate nucleus, MO = medulla oblongata.

pered the research directed to the understanding of the mechanism of action of ACTH–MSH-related peptides for inducing the stretching–yawning syndrome. Nevertheless, there have been a few reports showing that acetylcholine, opioid peptides, Ca^{2+} ions and nitric oxide are involved, because the stretching–yawning syndrome is prevented, partially or completely, by muscarinic receptor antagonists that cross the blood–brain barrier (Ferrari et al., 1963; Yamada and Furukawa, 1980) (see also Section 3 and references therein), by morphine (see Bertolini and Gessa, 1981 and references therein, and see also Section 8), by ω-conotoxin, a potent blocker of Ca^{2+} channels of the N-type, present in the nervous tissue, given into the lateral ventricles (Argiolas et al., 1990b), by organic Ca^{2+} channel blockers (Poggioli et al., 1993) and by nitric oxide synthase inhibitors (Poggioli et al., 1995).

In the last few years molecular biology studies have led to the cloning of ACTH–MSH receptor genes (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992), that also encode functional high-affinity receptors for ACTH–MSH peptides in several brain areas, including the hypothalamus, midbrain and brainstem (Gantz et al., 1993; Roselli-Rehfuss et al., 1993). Although the relationship between these receptors and the stretching–yawning syndrome is still unknown, this discovery is likely to reactivate the research aimed at understanding the mechanism responsible for the ACTH–MSH-induced stretching–yawning syndrome as well as for other central effects of these peptides.

3. Acetylcholine

Several lines of pharmacological evidence suggest that acetylcholine is involved in the expression of yawning. The evidence includes, for example, findings that cholinomimetic drugs (physostigmine, pilocarpine and others) induce yawning in rats (Urba-Holmgren et al., 1977; Yamada and Furukawa, 1980; Gower, 1987; Yamada et al., 1989; Zarrindstat and Poursoltan, 1989) and muscarinic receptor antagonists that cross the blood–brain barrier (atropine and scopolamine, but not methylscopolamine), but not nicotinic receptor antagonists (mecamylamine), prevent the yawning induced by ACTH–MSH peptides, dopamine D_2 receptor agonists and oxytocin (Ferrari et al., 1963; Urba-Holmgren et al., 1977; Yamada and Furukawa, 1980; Argiolas et al., 1986; Gower, 1987; Yamada et al., 1989; Zarrindstat and Poursoltan, 1989). More precise characterization of the involvement of the muscarinic M_1 and M_2 receptors in cholinomimetic-induced yawning comes from studies with recently developed muscarinic agents (see Fujikawa et al., 1996 and references therein). Many of the above studies, mainly those from the early eighties, suggested that yawning induced by dopamine D_2 receptor agonists was mediated by the activation of central cholinergic transmission, secondary to the inhibition of dopaminergic neurons by the stimulation of dopamine D_2 autoreceptors. However, in view of the results cited below that led to refutation of the hypothesis that dopamine D_2 receptor agonists induce yawning by acting on dopamine D_2 autoreceptors, the above interpretation needs to be re-evaluated. New findings that dopamine D_2 receptor agonists induce yawning by acting on postsynaptic dopamine D_2 receptors possibly located in the paraventricular nucleus of the hypothalamus (see Section 4 for details) need to be taken into account. As just mentioned, acetylcholine is also thought to be involved in the yawning response induced by ACTH–MSH and related peptides, because an increase in acetylcholine turnover was found to occur in the hippocampus during the ACTH-induced stretching–yawning syndrome (Wood et al., 1978, 1979) and because ACTH responses are prevented by selective antagonists of the muscarinic M_1 and M_2 receptors (Poggioli et al., 1991). Interestingly, yawning induced by dopamine D_2 receptor agonists, but not by cholinomimetic drugs, is prevented by medial septal lesions that interrupt the septo-hippocampal cholinergic pathway (Maeda et al., 1990; Melis et al., 1992a), supporting the possibility that dopamine D_2 receptor agonists induce yawning by activating postsynaptic dopamine D_2 receptors that, in turn, activate this cholinergic system. Unfortunately, this lesion also depletes the oxytocin content in the hippocampus (Melis et al., 1992a). Since oxytocin mediates dopamine D_2 receptor agonist-induced yawning, and oxytocin induces yawning when injected in the hippocampus (Melis et

al., 1986, 1989) (see also Sections 4 and 5), it is impossible to ascertain from the available data whether or not the septo-hippocampal cholinergic system is really involved in the yawning induced by dopamine D_2 receptor agonists. The major problem with the interpretation of the studies on the role of acetylcholine in the yawning response is that, while the study of Maeda et al., 1990 and Wood et al., 1978, 1979, suggest a role for the hippocampus, most other studies identified neither a possible site of action for the cholinomimetic agents in the induction of yawning, nor where the muscarinic receptor antagonists act to prevent the behavioral response induced by dopamine D_2 receptor agonists, oxytocin or ACTH–MSH-related peptides. Identification of these sites would certainly contribute to the understanding of the role of acetylcholine in the mediation of the yawning response induced by dopamine D_2 receptor agonists, oxytocin or ACTH–MSH peptides.

4. Dopamine

The involvement of dopamine in yawning was first suggested by the discovery that classical dopamine receptor agonists, i.e., apomorphine, *N*-*n*-propyl-norapomorphine, bromocriptine, lisuride, lergotrile, 3,4-dihydroxyphenylalanine (L-DOPA) and others, were able to induce yawning often together with penile erection in male rats (Mogilnicka and Klimek, 1977; Yamada and Furukawa, 1980; Baggio and Ferrari, 1983; Protais et al., 1983; Mogilnicka et al., 1984; Melis et al., 1987 and references therein). This finding was soon confirmed for other laboratory animals and extended to humans (see Lal et al., 1989 and references therein). As with yawning induced by ACTH–MSH peptides (see Section 2), dopamine receptor agonist-induced yawning is abolished by hypophysectomy, but not by neonatal monosodium glutamate treatment, which depletes brain opiomelanocortin peptides. These findings suggest that peripheral and central ACTH–MSH peptides are not involved in the yawning response to dopamine receptor agonists (see Serra et al., 1983a,b; Argiolas et al., 1987a; Melis et al., 1994a). Dopamine receptor agonist-induced yawning is also abolished by castration, a treatment that also abolishes the penile erection induced by these agents. In castrated male rats, the yawning response to dopamine receptor agonists is partially restored by chronic replacement therapy with estradiol benzoate alone or with the combination of estradiol benzoate plus 5-hydroxy-testosterone. Testosterone given alone, however, while it restores penile erection in these same animals, does not restore yawning (see Melis et al., 1994a). This suggested that sexual steroids, in particular estrogens, exert a permissive role on dopamine receptor agonist-induced yawning and that the neuronal circuits mediating the yawning response can be differentiated from those that mediate the erectile response, although the two responses often occur concomitantly. That estrogens influence dopamine D_2 receptor agonist-induced yawning differently from testosterone is also supported by the completely different effect of these sex steroids on yawning in intact male rats when compared to castrated male rats. In intact male rats, estrogens inhibit dopamine receptor agonist-induced yawning, while testosterone is ineffective, although a role for testosterone, which can be transformed into estrogen plus 5-hydroxy-testosterone by brain aromatase enzymatic activity, cannot be ruled out (see Berendsen and Gower, 1986; Melis et al., 1994a). The inhibitory effect of estrogens on yawning induced by dopamine receptor agonists, suggested that the higher levels of circulating estrogens are responsible for the weaker effect of dopamine receptor agonists on the yawning response in intact female rats when compared to that in male rats (Serra et al., 1984).

With the discovery of the existence of two main classes of dopamine receptors (e.g. the D_1 and D_2 receptors) (see Kebabian and Calne, 1982; Stoof and Kebabian, 1984), the dopamine receptor agonist-induced yawning response was soon found to be mediated by the stimulation of dopamine receptors of the D_2 type, i.e. the response was induced by selective dopamine D_2 receptor agonists, for instance quinpirole (LY 171555) but not by selective dopamine D_1 receptor agonists, i.e., *R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol (SKF 38393) (see Melis et al., 1987 and references therein). Since most dopamine D_2 receptor agonists induce yawning when given at very low doses, but not at high doses that induce hypermotility and stereotyped behaviors that mask the yawning response, early results suggested that this response was due to the stimulation of dopamine D_2 autoreceptors (Mogilnicka and Klimek, 1977; Yamada and Furukawa, 1980; Dourish et al., 1985; Serra et al., 1986 and references therein), that is, of receptors located on the cell bodies and/or in the synapses of dopaminergic neurons, whose stimulation inhibits neuronal activity (Di Chiara et al., 1976). It was also suggested that dopaminergic neurons, whose inhibition was responsible for the induction of yawning by dopamine D_2 receptor agonists, were nigrostriatal dopaminergic neurons since bilateral lesions of the nigrostriatal dopaminergic system by 6-hydroxydopamine prevent dopamine receptor agonist-induced yawning in rats (Dourish and Hutson, 1985; Stoessl et al., 1987 and references therein). This hypothesis was also supported by results of microinjection studies showing that the injection of high doses of dopamine receptor agonists in the striatum or the septum induce yawning in rats (Dourish et al., 1985; Yamada et al., 1986; Okuyama et al., 1987). However, more recent findings made the above hypotheses untenable. First, the administration of selective dopamine D_2 autoreceptor agonists was found to be unable to induce yawning (Stahle and Ungerstedt, 1984). Second, microinjection studies revealed that apomorphine and other dopamine D_2 receptor agonists induce yawning when injected in nanogram amounts in the paraventricular nucleus of the hypothala-

mus and not in the striatum (Melis et al., 1987). Third, the yawning response to dopamine D_2 receptor agonists, given systemically or into the paraventricular nucleus, was found to be prevented not only by selective dopamine D_2 receptor antagonists, i.e., L-sulpiride, but also by the selective dopamine D_1 receptor antagonist SCH 23390 (*R*-(+)-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5,-tetrahydro1H-3-benzazepine), which presumably acts on postsynaptic D_1 receptors (Morelli et al., 1986; Melis et al., 1987; Serra et al., 1987). Fourth, no correlation was found between the yawning response and the extracellular levels of dopamine and its metabolites in the striatum, as measured by microdialysis in vivo (for a review on this subject see Stahle, 1992). The above results led to the suggestion that dopamine D_2 receptor agonists induce yawning when injected in high doses in the striatum or the septum because of their diffusion through the very nearby lateral ventricles, across the brain to the paraventricular nucleus of the hypothalamus, where they induce yawning by stimulating postsynaptic dopamine D_2 receptors (Melis et al., 1987).

In this regard, it is noteworthy that this hypothalamic nucleus contains dopaminergic nerve endings that belong to the incerto-hypothalamic dopaminergic system. These neurons have their cell bodies in the hypothalamic A13 and A14 cell groups, branch extensively and innervate several hypothalamic nuclei, including the paraventricular nucleus (for a detailed description of the anatomy of incerto-hypothalamic dopaminergic neurons see Lindvall and Björklund, 1978). In agreement with the above hypothesis, dopamine D_2 receptor agonist-induced yawning was prevented by bilateral electrolytic lesions of the paraventricular nucleus (Argiolas et al., 1987b). This finding was explained by assuming that dopamine D_2 receptor agonists induce yawning by activating oxytocinergic neurons projecting to extra-hypothalamic brain areas (see also Section 5). It was consistent with this possibility that apomorphine-induced yawning was prevented in a dose-dependent manner by oxytocin receptor antagonists with a potency that paralleled the potency of these compounds to block oxytocinergic receptors (Melis et al., 1989). Furthermore, immunocytochemical studies have shown that dopaminergic synapses impinge on the cell bodies of oxytocinergic neurons in the paraventricular nucleus (Buijs et al., 1984; Lindvall et al., 1984). Together, all the above results suggested that a dopamine–oxytocin link was involved in the expression of yawning at the paraventricular level. More precisely, it was suggested that dopamine receptor agonists induce yawning by stimulating dopamine D_2 receptors located in the cell bodies of oxytocinergic neurons projecting to extra-hypothalamic brain areas, thereby releasing oxytocin in brain areas distant from the paraventricular nucleus (for a review of central oxytocinergic projections see Argiolas and Gessa, 1991). Among these areas, the hippocampus is certainly one of the most important candidates, since electrolytic lesions of the medial septum, which deplete hippocampal oxytocin, prevent apomorphine-induced yawning (Melis et al., 1992a). Also, oxytocin injected in the CA1 field of the hippocampus induces yawning (Melis et al., 1986). The importance of the hippocampus in dopamine receptor agonist-induced yawning was emphasised by the finding that apomorphine increases the oxytocin concentration, not only in plasma but also in the hippocampus of male rats (Melis et al., 1990). However, it is not ruled out that other brain areas receiving oxytocinergic projections from the paraventricular nucleus might be involved as well (Melis et al., 1992a).

One possible mechanism by means of which dopamine D_2 receptor agonists increase oxytocinergic transmission in the paraventricular nucleus of the hypothalamus to induce yawning is that these compounds increase the intracellular Ca^{2+} concentration in the cell bodies of oxytocinergic neurons mediating this behavioral response. The intracellular Ca^{2+} increase apparently activates nitric ox-

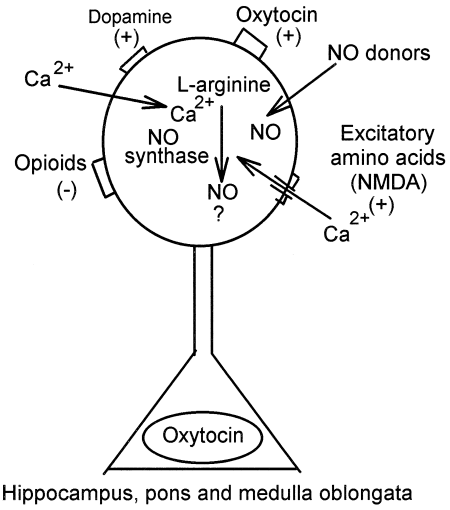

Fig. 1. Schematic representation of a hypothetical mechanism of action by means of which oxytocinergic neurons projecting to extra-hypothalamic brains areas mediate the expression of yawning induced by several neurotransmitters and/or neuropeptides in the paraventricular nucleus of the hypothalamus. 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 projecting to extra-hypothalamic brain areas the first two by stimulating specific receptors coupled to ω-conotoxin-sensitive Ca^{2+} channels by a pertussis toxin-sensitive G_o/G_q protein and the second 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 nitric oxide synthase. Nitric oxide formed endogenously (or derived by nitric oxide donors) in turn would activate 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 paraventricular nucleus, i.e. the hippocampus, the pons and/or the medulla oblongata. The mechanism by means of which opioids inhibit oxytocinergic transmission is still unknown, but evidence showing that opioid receptors might be located in the paraventricular oxytocinergic cell bodies as well as that their activation prevents the activation of nitric oxide synthase by dopamine, oxytocin and excitatory amino acids have been provided (see text for details).

ide synthase to produce nitric oxide that, in turn, activates oxytocinergic transmission (see also Sections 5 and 7 for details). If one assumes that this hypothesis is correct, paraventricular dopamine D_2 receptors would be coupled through a G protein directly to a Ca^{2+} channel or to phospholipase C that converts phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate (IP_3), which mobilizes intracellular Ca^{2+} as already described for other neuronal tissues (for a review of the signal transduction systems coupled to dopamine receptors see Baldessarini, 1996 and references therein). It is consistent with this hypothesis that apomorphine-induced yawning is prevented by pertussis toxin, that inhibits the activity of several G proteins (see Stancampiano et al., 1992 and references therein), by nanogram amounts of ω-conotoxin, a potent blocker of Ca^{2+} channels of the N-type present in neuronal tissue (Argiolas et al., 1990a), by organic Ca^{2+} channel blockers (Argiolas et al., 1989a), or by nitric oxide synthase inhibitors, given in the paraventricular nucleus of the hypothalamus (Melis et al., 1994c) (Fig. 1).

5. Oxytocin

Simultaneously with the discovery that dopamine D_2 receptor agonists act in the paraventricular nucleus of the hypothalamus to induce yawning, it was found that oxytocin, the neurohypophysial peptide with a hormonal role in parturition and lactation, was extremely potent to induce yawning associated with penile erection in several laboratory animals when injected in the central nervous system (Argiolas et al., 1986). The oxytocin response was abolished by hypophysectomy and by castration, but not by neonatal monosodium glutamate (Argiolas et al., 1989b; Melis et al., 1994a). In castrated rats, oxytocin-induced yawning was restored by replacement therapy with estradiol benzoate, or with estradiol benzoate plus 5-hydroxytestosterone, but not with testosterone alone (Melis et al., 1994a). Testosterone was also ineffective to restore oxytocin-induced yawning in hypophysectomized rats (Argiolas et al., 1989b). It was soon discovered that oxytocin also was extremely effective to induce yawning when injected in the paraventricular nucleus of the hypothalamus. It induced the response in more than 60% of the rats treated, even when injected unilaterally in this nucleus at a dose as low as 3 ng (3 pmol) (Melis et al., 1986). Oxytocin was found to induce yawning also when injected in the CA1 field of the hippocampus, but only when injected bilaterally and at doses higher than those active when injected in the paraventricular nucleus (Melis et al., 1986). The importance of the paraventricular nucleus in the expression of yawning induced by oxytocin was made clear by the fact that bilateral electrolytic lesions of the nucleus, which almost completely deplete the brain oxytocin content (Lang et al., 1983; Hawthorn et al., 1985), prevented not only the yawning induced by oxytocin but also that induced by dopamine D_2 receptor agonists (Argiolas et al., 1987b) (see also Section 4). In contrast, these lesions did not prevent ACTH-induced yawning.

Structure–activity relationship studies revealed that oxytocin effect was mediated by uterine-type oxytocinergic receptors (Argiolas et al., 1989c). Only the intact oxytocin molecule, or oxytocin peptide analogs with amino acid substitutions preserving the biological activity of the peptide on the uterus and/or the mammary gland, were active to induce yawning. In contrast, removal of the C terminal amino acid, $GlyNH_2$, decreased 100-fold the potency of the peptide to induce yawning, while removal of the C terminal tripeptide, Pro–Leu–$GlyNH_2$, abolished the activity. The latter result was similar to the result of opening the disulfide bridge between Cys^1 and Cys^6. Conversely, oxytocin-induced yawning was prevented by potent oxytocin nonapeptide receptor antagonists given into the lateral ventricles or into the paraventricular nucleus with a potency that was parallel to the potency of these compounds to block oxytocinergic receptors in the uterus and/or the mammary gland (Argiolas et al., 1987c; Melis et al., 1989). The above results suggested that, when injected in the paraventricular nucleus, oxytocin induces yawning by activating its own transmission. This explanation had already been offered for the synchronous activation of magnocellular oxytocinergic neurons, that mediates the massive milk ejection induced by suckling during lactation (Freund-Mercier and Richard, 1981; Poulain and Wakerly, 1982; Moss et al., 1984). One of the oxytocinergic pathways activated by oxytocin itself, that projects to extra-hypothalamic brain areas and whose activation causes yawning, might be the pathway referred to above, that projects to the hippocampus and that mediates dopamine D_2 receptor agonist-induced yawning. However, other pathways, for instance those projecting to the pons and/or the medulla oblongata, might be involved as well. For example, lesions of the medial septum, that deplete hippocampal oxytocin and prevent apomorphine-induced yawning, are unable to prevent the oxytocin-induced response (see Melis et al., 1992a and references therein).

Among possible mechanisms by means of which oxytocin activates its own transmission in the paraventricular nucleus to induce yawning, one is that by which oxytocin increases intracellular Ca^{2+} concentration in the cell bodies of its neurons mediating the behavioral response, as suggested for dopamine D_2 receptor agonists. The intracellular Ca^{2+} increase apparently activates nitric oxide synthase, to produce nitric oxide that, in turn, activates oxytocinergic transmission (see Section 7 for details). Hence, paraventricular oxytocin receptors also, like dopamine D_2 receptors, would be coupled through a G protein directly to Ca^{2+} channels or to phospholipase C that converts phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate (IP_3), that mobilizes intracellular Ca^{2+}, as found in the uterus and/or in

the mammary gland (see Argiolas and Gessa, 1991 and references therein). Interestingly, an increase in intracellular Ca^{2+} was also found in isolated supraoptic cells activated by oxytocin (Lambert et al., 1994). In agreement with this latter hypothesis, oxytocin-induced yawning is prevented by pertussis toxin that inhibits several G proteins (see Stancampiano et al., 1992 and references therein), by nanogram amounts of ω-conotoxin, that blocks neuronal Ca^{2+} channels (Argiolas et al., 1990a), or by organic Ca^{2+} channel blockers (Argiolas et al., 1989d) or by nitric oxide synthase inhibitors (Melis et al., 1994c) when injected in the paraventricular nucleus (Fig. 1).

6. Excitatory amino acids

The studies reviewed above suggest that there are oxytocinergic neurons originating in the paraventricular nucleus and projecting to extra-hypothalamic brain areas, i.e. the hippocampus or the medulla oblongata, whose activation by dopamine D_2 receptor agonists or by oxytocin itself is responsible for the expression of yawning. The paraventricular nucleus of the hypothalamus, however, has not only nerve endings containing dopamine but also many other neurotransmitters and neuropeptides (for a review of the organization of the paraventricular nucleus see Swanson and Swachensko, 1983 and references therein). This raised the possibility that other neurotransmitters and/or neuropeptides in this nucleus would influence the expression of yawning by modulating oxytocinergic transmission. Indeed, the discovery followed that excitatory aminoacids also are involved in the control of yawning at the paraventricular level. *N*-Methyl-D-aspartic acid (NMDA), a potent and selective excitatory amino acid receptor agonist of the NMDA receptor subtype, but not (±)-α-(amino)-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) or *trans*-(±)-1-amino-1,3-cyclopentane-dicarboxylic acid (APCD), selective agonists of the excitatory amino acid receptors of the AMPA and metabotropic receptor subtypes, respectively, (see Monaghan et al. (1989) for the classification of excitatory amino acid receptors) induced yawning when injected in nanogram amounts in the paraventricular nucleus (Melis et al., 1994b). The NMDA response was prevented not only by dizocilpine (MK-801), a potent non-competitive antagonist of NMDA receptors, as expected, but also by [d$(CH_2)_5$Tyr(Me)^2-Orn^8]vasotocin, a potent oxytocin receptor antagonist when given into the lateral ventricles but not into the paraventricular nucleus (Melis et al., 1994b). Since dizocilpine injected in the paraventricular nucleus was unable to prevent apomorphine- or oxytocin-induced yawning, while it prevented the NMDA response (Melis et al., 1992b, 1994b), the above results are in line with the hypothesis that NMDA also induces this behavioral response by activating oxytocinergic transmission in the hypothalamic nucleus, as do dopamine D_2 receptor agonists and oxytocin itself (Fig. 1). It is also consistent with this hypothesis that haloperidol, that blocks dopamine receptors, and [d$(CH_2)_5$Tyr(Me)^2-Orn^8]vasotocin, that blocks oxytocin receptors, given in the paraventricular nucleus, do not prevent NMDA-induced yawning, while they do prevent dopamine D_2 receptor agonist- and oxytocin-induced yawning, respectively. This, then, rules out the possibility that NMDA induces the behavioral response by releasing dopamine or oxytocin in the paraventricular nucleus (Melis et al., 1994b).

As to the mechanism through which NMDA induces yawning, via activation of oxytocinergic transmission in the paraventricular nucleus, the easiest explanation is that NMDA increases the intracellular Ca^{2+} concentration in the cell bodies of oxytocinergic neurons mediating this behavioral response by increasing the Ca^{2+} influx through the Ca^{2+} channels coupled to NMDA receptors (Monaghan et al., 1989). The intracellular Ca^{2+} increase activates nitric oxide synthase, to produce nitric oxide, thus stimulating oxytocinergic transmission (see Section 7 for details). It is consistent with this explanation that NMDA-induced yawning is prevented by nitric oxide synthase inhibitors given in the paraventricular nucleus (Melis et al., 1994c). Also, a mechanism similar to this one is unanimously accepted to explain the activation by NMDA of guanylate cyclase in other brain tissues (Snyder, 1992; Southam and Garthwaite, 1993; Schuman and Madison, 1994) (Fig. 1).

7. Nitric oxide

The key role of the paraventricular nucleus in the expression of yawning as just described, has recently been confirmed by the finding that nitric oxide, a neurotransmitter/neuromodulator in several tissues including the central nervous system (Ignarro, 1990; Snyder, 1992; Moncada and Higgs, 1993; Southam and Garthwaite, 1993; Schuman and Madison, 1994), is also involved at the paraventricular level in the expression of yawning, at least when it is induced by dopamine D_2 receptor agonists, NMDA and oxytocin. The paraventricular nucleus is one of the brain areas richest in nitric oxide synthase, the Ca^{2+}-calmodulin-dependent enzyme that forms, in neurons, nitric oxide from L-arginine (Bredt and Snyder, 1990; Snyder, 1992; Vincent and Kimura, 1992; Southam and Garthwaite, 1993; Schuman and Madison, 1994). It is also highly relevant that the enzyme is also present in oxytocinergic neurons (Bredt et al., 1990; Torres et al., 1993; Sanchez et al., 1994) in addition to corticotropin-releasing hormone-, vasopressin- and somatostatin-containing neurons (see Amir, 1995). A facilitatory role of nitric oxide in the control of yawning was first suggested by the ability of nitric oxide synthase inhibitors (e.g. N^G-nitro-L-arginine methylester, N^G-monomethyl-L-arginine and N^G-monomethyl-D-arginine) injected into the lateral ventricles as well as in the paraventricular nucleus to prevent the yawn-

ing induced by dopamine receptor agonists, NMDA and oxytocin with a potency parallel to their potency for inhibition of nitric oxide synthase (Melis and Argiolas, 1993; Melis et al., 1994c,d). The fact that the inhibitory effect of nitric oxide synthase inhibitors on yawning was prevented by the prior administration of L-arginine, the precursor of nitric oxide, into the paraventricular nucleus, further confirmed this role (Melis and Argiolas, 1997). That classical nitric oxide donors also, such as nitroglycerin, sodium nitroprusside, hydroxylamine and others, injected in the paraventricular nucleus, induce yawning, apparently by activating central oxytocinergic transmission, this response being prevented by the potent oxytocin nonapeptide receptor antagonist, [d(CH_2)$_5$Tyr(Me)^2-Orn^8]vasotocin, given into the lateral ventricles, but not in the paraventricular nucleus (Melis and Argiolas, 1995, 1997; Melis et al., 1995b) is also evidence for the key role of nitric oxide.

Results of the above studies suggested that dopamine D_2 receptor agonists, NMDA and oxytocin induce yawning by activating nitric oxide synthase in the paraventricular nucleus (Fig. 1). This possibility was confirmed by showing that dopamine D_2 receptors agonists, NMDA and oxytocin, in doses that induce yawning, increase nitric oxide production, that is, nitric oxide synthase activity (measured by in vivo microdialysis) in the paraventricular nucleus (Melis et al., 1996, 1997a,c; Melis and Argiolas, 1997). In these studies, paraventricular dialysate was obtained with a vertical microdialysis probe inserted in the paraventricular nucleus of male rats. After a 2 h perfusion with a physiological Ringer's solution, a dose of apomorphine, NMDA or oxytocin that induced yawning was given and the dialysate was collected in aliquots of 40 μl every 20 min. Since nitric oxide cannot be measured directly by this method because of its very short half-life (1–5 s) (Ignarro, 1990), the concentration of the reaction products, nitrite (NO_2^-) and nitrate (NO_3^-), of nitric oxide with oxygen were measured in the paraventricular dialysate. The concentration of these ions has been shown to be a reliable indicator of nitric oxide synthase activity in biological fluids, when red blood cells are absent, as in extracellular brain dialysate (Ignarro, 1990; Luo et al., 1993; Ohta et al., 1994; Melis et al., 1996, 1997a,c). In agreement with the above hypothesis, the ability of dopamine D_2 receptor agonists, NMDA and oxytocin to increase nitric oxide production in the paraventricular nucleus was prevented, as expected, by selective receptor antagonists of the above drugs. These antagonists also prevent the behavioral response induced by the above compounds. That is, haloperidol prevents the dopamine receptor agonist-induced responses, dizocilpine (MK-801) prevents NMDA responses and [d(CH_2)$_5$Tyr(Me)^2-Orn^8]-vasotocin prevents the responses to oxytocin (Melis et al., 1996, 1997a,c). Most important, the yawning and the increase of nitric oxide production were also prevented by N^G-nitro-L-arginine methylester, a potent nitric oxide synthase inhibitor (Fig. 2). Conversely, the increase in NO_2^- concentration that the above compounds induced in the paraventricular dialysate was prevented by hemoglobin, a potent nitric oxide scavenger. A problem with the above interpretation is, however, that hemoglobin, despite its ability to prevent the NO_2^- increase, did not prevent the behavioral response to apomorphine, NMDA or oxytocin (see also Melis et al., 1994c, 1995b) (Fig. 2) as well as that to nitric oxide donors (Melis and Argiolas, 1995). This discrepancy might be explained by assuming that nitric oxide produced by the activation of dopamine, NMDA and oxytocin receptors or by nitric oxide donors facilitates the expression of yawning by acting intracellularly as a second messenger, that is, inside the oxytocinergic neurons in which it is formed and that mediate yawning, rather than as a neurotransmitter after being released in the extracellular space. Hemoglobin would, indeed, be expected to scavenge only nitric oxide that is released in the extracellular space, because its high molecular weight makes it unable to cross cellular membranes. This does not rule out the possibility that nitric oxide released from oxytocinergic neurons acts as a neurotransmitter, for instance to mediate other hypothalamic effects of dopamine D_2 receptor agonists, NMDA or oxytocin itself (for a review of the nitric oxide functions in the hypothalamus see Amir, 1995).

The mechanism by which nitric oxide activates oxytocinergic transmission in the paraventricular nucleus to induce yawning is unknown. Current information suggests that guanylate cyclase, one of the best known targets of nitric oxide (Schuman and Madison, 1994 and references therein), is apparently not involved, at least in the paraventricular nucleus. Of the experimental evidence supporting this hypothesis, the most important is the inability of 8-bromo-cyclic guanosine 3′,5′-monophosphate (c-GMP), a stable phosphodiesterase-resistant analog of c-GMP, to induce yawning when injected in the paraventricular nucleus (Melis and Argiolas, 1995). Also arguing against a role of guanylate cyclase in the paraventricular nucleus is the inability of methylene blue and 6-anilino-quinoline-5,8-quinone (LY 83583), putative inhibitors of guanylate cyclase, to prevent apomorphine-, oxytocin-, NMDA- and nitric oxide donor-induced yawning when they are given in the paraventricular nucleus (Melis et al., 1994c,d, 1995b; Melis and Argiolas, 1995). Methylene blue also does not prevent the paraventricular NO_2^- increase induced by all the above compounds (Melis et al., 1996, 1997a,c) (Fig. 2). It is pertinent to recall that other targets of nitric oxide in addition to guanylate cyclase have been identified (for a review of the targets of nitric oxide see Schuman and Madison, 1994). However, since methylene blue prevents the yawning induced by all the above compounds when it is given into the lateral ventricles (Melis et al., 1994c,d, 1995b; Melis and Argiolas, 1995), a role of guanylate cyclase at sites other than the paraventricular nucleus in the expression of this behavioral response cannot be ruled out.

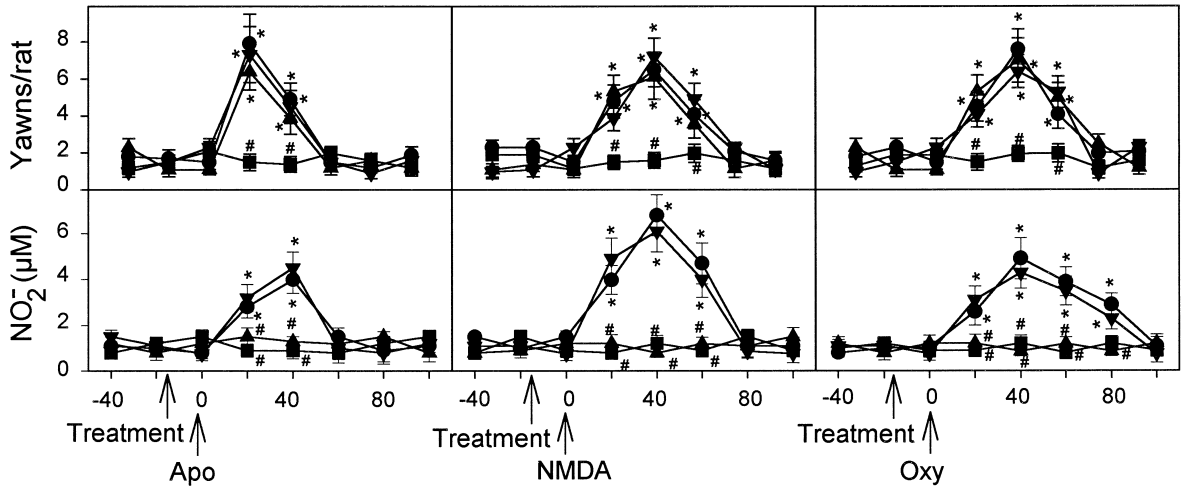

Fig. 2. Apomorphine, NMDA and oxytocin induce yawning and increase NO_2^- concentration in the paraventricular dilaysate obtained with a vertical microdialysis probe implanted in the paraventricular nucleus of the hypothalamus of freely moving male rats: effect of nitro-L-arginine methylester, hemoglobin and methylene blue. Rats implanted with a vertical microdialysis probe directed to the paraventricular nucleus and/or a cannula for microinjection into the lateral ventricles or in the paraventricular nucleus were placed individually in a Plexiglas cage and perfused with a Ringer's solution. Apomorphine (80 μg/kg s.c.), NMDA (50 ng in the paraventricular nucleus) and oxytocin (30 ng into the lateral ventricles) were given after a 120 min equilibration period of the probe with the perfusion buffer (time = 0). Saline (0.3 μl) (circles), nitro-L-arginine methylester (20 μg) (squares), hemoglobin (20 μg) (triangles) or methylene blue (20 mg) (up-side-down triangles) were all given in the paraventricular nucleus 15 min before apomorphine, NMDA or oxytocin 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 (Melis et al., 1996). During the perfusion, rats were observed to count yawning episodes. Each value is the mean ± S.E.M. of 6 rats found to have the tip of the probe in the paraventricular nucleus as determined by histological analysis. $^*P < 0.01$ with respect to the pretreatment values (negative times); $^\#P < 0.01$, with respect to the values of the corresponding groups treated with apomorphine, NMDA or oxytocin (one-way ANOVA followed by Duncan's multiple range test).

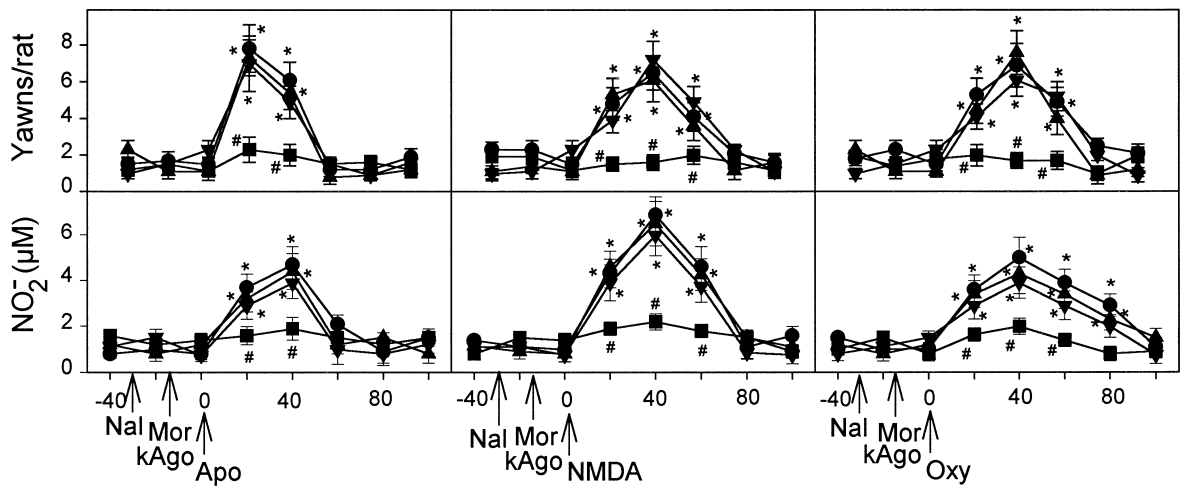

Fig. 3. Effect of morphine and U-69,593 on apomorphine-, NMDA- and oxytocin-induced yawning and NO_2^- concentration increase in the paraventricular dilaysate obtained with a vertical microdialysis probe implanted in the paraventricular nucleus of the hypothalamus of freely moving male rats: reversal by naloxone. Rats implanted with a vertical microdialysis probe directed at the paraventricular nucleus were used. The experimental conditions were identical to those reported in the legend of Fig. 2. Apomorphine (80 μg/kg s.c.), NMDA (50 ng in the paraventricular nucleus) and oxytocin (30 ng into the lateral ventricles) were given after a 120 min equilibration period of the probe with the perfusion buffer (time = 0). Saline (0.3 μl) (circles) morphine (10 μg) (squares) and U 69,593 (10 μg) (triangles) were given 15 min before apomorphine, NMDA or oxytocin. Naloxone (3 mg/kg i.p.) (up-side-down triangles) was given 15 min before morphine. The perfusion rate was 2 μl/min. Aliquots of 40 μl were collected every 20 min and analyzed for NO_2^- content (Melis et al., 1996). During the perfusion, rats were observed to count yawning episodes. Each value is the mean ± S.E.M. of 6 rats found to have the tip of the probe in the paraventricular nucleus as determined by histological analysis. $^*P < 0.01$ with respect to the pretreatment values (negative times); $^\#P < 0.01$, with respect to the values of the corresponding groups treated with apomorphine, NMDA or oxytocin (one-way ANOVA followed by Duncan's multiple range test).

8. Opioid peptides

The studies mentioned above show that dopamine, excitatory amino acids, and oxytocin facilitate the expression of yawning, apparently by increasing nitric oxide production in the cell bodies of oxytocinergic neurons, in turn causing an increase of central oxytocinergic transmission. Interestingly, the paraventricular nucleus contains endogenous opioid peptides and receptors (O'Donohue and Dorsa, 1982 and references therein) that exert an inhibitory control on oxytocinergic transmission (Muhlethaler et al., 1980; Pittman et al., 1980; Wuarin et al., 1988). Opiate drugs, such as morphine, that stimulate opioid receptors are very effective to prevent the yawning induced by ACTH–MSH-related peptides, dopamine receptor agonists and oxytocin (Ferrari et al., 1963; Argiolas et al., 1986; Berendsen and Gower, 1986). Conversely, the opioid receptor antagonist, naloxone, has been reported to increase the yawning induced by dopamine receptor agonists and ACTH, but the effect was not dose-dependent (Bertolini et al., 1978; Berendsen and Gower, 1986). This led us to investigate the possibility that opiate drugs prevent yawning induced by dopamine D_2 receptor agonists, NMDA and oxytocin by acting in the paraventricular nucleus. As expected in this case, morphine injected in the paraventricular nucleus was very effective to prevent the yawning induced by dopamine D_2 receptor agonists, NMDA and oxytocin in a dose-dependent manner (Melis et al., 1992c, 1997d) (Fig. 3). These findings suggested that endogenous opioid peptides exert an inhibitory control at the paraventricular level on the yawning response, possibly by inhibiting central oxytocinergic transmission. It is consistent with this that yawning is one of the most common signs of the opiate withdrawal syndrome in opiate addicts (O'Brien, 1996). Apparently, morphine acts to prevent yawning induced by dopamine D_2 receptor agonists, by oxytocin or by NMDA through stimulation of opioid receptors of the μ type, since the morphine effect is prevented by the prior administration of naloxone, that blocks opioid receptors, while U-69,593, an opioid receptor agonist 500 times more potent than morphine on the κ opioid receptor subtype, is ineffective (Melis et al., 1992c, 1997d) (Fig. 3).

Opioid receptor stimulation may prevent the yawning induced by dopamine receptor agonists, oxytocin and NMDA, through a mechanism related to a concomitant decrease in the production of nitric oxide induced by the above compounds, in analogy to morphine-induced prevention of yawning. The decrease was measured by in vivo microdialysis and was found to occur concomitantly with the prevention of the behavioral response (Melis et al., 1997b,d) (Fig. 3). The prevention by morphine of the nitric oxide production increase 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 nitric oxide synthase activity. Although the molecular mechanisms by means of which stimulation of μ opioid receptors causes a decreased Ca^{2+} influx is unknown, the finding suggests that opioids and nitric oxide have opposite effects on the expression of yawning at the paraventricular level.

Morphine is also very effective in preventing the stretching–yawning syndrome induced by ACTH–MSH peptides (Ferrari et al., 1963). However, since this ACTH–MSH response is apparently mediated by neuronal systems different from the oxytocinergic neurons mediating the yawning induced by dopamine receptor agonists, oxytocin and NMDA, further studies are necessary to identify the site(s) and/or the mechanism of action of morphine for prevention of the stretching–yawning syndrome induced by the peptides. It is noteworthy that this ACTH–MSH-induced response is also prevented by Ca^{2+} channel blockade and by nitric oxide synthase inhibitors (Argiolas et al., 1990b; Poggioli et al., 1993, 1995), raising the possibility that ACTH–MSH peptides also, like dopamine receptor agonists, oxytocin and NMDA, exert effects opposite to those of morphine on the Ca^{2+} influx through Ca^{2+} channels in neurons involved in the expression of the yawning induced by ACTH–MSH peptides, as suggested above for the paraventricular oxytocinergic neurons that mediate the yawning response induced by the above compounds.

9. Serotonin

The assumption of serotonin (5-HT) involvement in the expression of yawning is also based on pharmacological experiments showing that drugs, such as 1-(3-chlorophenyl)-piperazine (mCPP) and *N*-(3-trifluoro-methylphenyl)-piperazine (TFMPP) (for a review of the classification of 5-HT receptors see Humprey et al., 1993 and references therein) which act selectively on the 5-HT_{2C} receptor subtype, are extremely effective to induce yawning when given systemically not only in rats, but also in monkey and humans (Berendsen and Broekkamp, 1987; Szele et al., 1988; Berendsen et al., 1990; Stancampiano et al., 1994). The 5-HT_{2C} receptor agonist-induced yawning is prevented, not only by the blockade of these receptors, but also by stimulation of 5-HT_{1A} and 5-HT_2 receptors, that also prevents dopamine receptor-induced yawning (Gower et al., 1986; Berendsen and Broekkamp, 1987; Simon et al., 1992). This suggests that 5-HT_{1A} and 5-HT_2 receptors exert an inhibitory control on yawning opposite to that of 5-HT_{2C} receptors and that a complicated dopamine–serotonin interaction is involved in the expression of the yawning response. Interestingly, the paraventricular nucleus receives a dense 5-HT projection originating in the dorsal raphe nuclei (Van de Kar, 1991) and 5-HT receptor agonists increase the plasma oxytocin concentration (Saydoff et al., 1991; Bagdy et al., 1992; Bagdy and Kalogeras, 1993), raising the possibility that 5-HT_{2C} receptor agonists also induce yawning by acting at the

paraventricular level by activating oxytocinergic transmission. However, findings acquired so far argue against this possibility. First, 5-HT_{2C} receptor agonists (e.g. mCPP and TFMPP), unlike dopamine D_2 receptor agonists, NMDA and oxytocin, do not induce yawning when they are injected directly in the paraventricular nucleus. Second, the yawning induced by 5-HT_{2C} receptor agonists, unlike that induced by dopamine D_2 receptor agonists, NMDA or oxytocin itself, is not prevented by oxytocin receptor antagonists given into the lateral ventricles. Third, 5-HT_{2C} receptor antagonists do not prevent dopamine D_2 receptor agonist- or oxytocin-induced yawning (Stancampiano et al., 1994). Despite the above differences between yawning induced by dopamine D_2 receptor agonists, NMDA, oxytocin and 5-HT_{2C} receptor agonists, the 5-HT_{2C} receptor agonist-induced yawning is also prevented by nitric oxide synthase inhibitors given into the lateral ventricles (Melis et al., 1995a). Since nitric oxide synthase inhibitors also prevent ACTH-induced yawning (Poggioli et al., 1995), there is further evidence that nitric oxide is also involved in the control of yawning in areas other than the paraventricular nucleus.

10. Other neurotransmitters and neuropeptides

A few studies have shown that drug-induced yawning can also be influenced by drugs that act on other neuronal systems, i.e. GABA (γ-amminobutyric acid), noradrenaline and neurotensin, suggesting a role for these three in the expression of yawning. GABA-transaminase inhibitors have been reported to increase yawning frequency in rats treated with physostigmine but not with apomorphine, while baclofen inhibits cholinomimetic- and apomorphine-induced yawning (Doger et al., 1989). These findings led the latter authors to suggest that $GABA_B$ receptors inhibit the yawning response by modulating acetylcholine transmission, considered to be under inhibitory dopaminergic control (see Acetylcholine section). Drugs that act on α- and β-adrenoceptors also have been found able to modify the yawning response when it is induced by dopamine D_2 receptor agonists, cholinomimetic agents, ACTH–MSH peptides or oxytocin. The yawning response thus induced was usually inhibited by α-adrenoceptor antagonists and potentiated by β-adrenoceptor antagonists, although some of the findings are controversial, especially regarding the role of the α_1- and α_2-adrenoceptor subtypes (Baraldi and Ferrari, 1980; Poggioli et al., 1984; Gower et al., 1986; Yamada et al., 1989; Fujikawa et al., 1995; Kimura et al., 1996).

Finally, the neuropeptides, neurotensin and luteinizing hormone–releasing hormone (LH-RH), have been reported to antagonize drug-induced yawning. Neurotensin antagonizes apomorphine- and pilocarpine-induced yawning in male rats, an effect shared with higher potency with one of its enkephalinase-resistant analogs, [D-Trp^{11}]-neurotensin, when it is given into the lateral ventricles (Nouel and Costentin, 1991). This effect was explained by assuming that neurotensin is a type of endogenous neuroleptic, that interacts in an inhibitory fashion with the dopaminergic systems mediating yawning. Luteinizing hormone-releasing hormone, like neurotensin, was found to prevent apomorphine-induced yawning when given subcutaneously. This effect was assumed to be secondary to the peptide's modulation of the sensitivity of central dopamine receptors mediating yawning (Mora and Diaz-Veliz, 1989). However, none of the studies just cited provided information about the brain sites in which GABAergic and noradrenergic agents, neurotensin and luteinizing-hormone-releasing hormone act to alter the yawning response. Also, neither experimental demonstration not detailed analysis of the mechanisms supposed to be responsible for the effect of these various agents on yawning are available. Further studies are necessary to clarify the exact role of GABA, noradrenaline, neurotensin and luteinizing hormone-releasing hormone in the production of yawning.

Finally, yawning has been observed in rats after the systemic injection of ovine prolactin (Laping and Ramirez, 1986). This response was assumed to be mediated by the release of dopamine in the nigrostriatal system, inhibiting dopaminergic neurons by acting on dopamine D_2 autoreceptors (Laping and Ramirez, 1988). However, in view of the more recent findings reviewed here, which seem to rule out an involvement of dopamine autoreceptors in the expression of yawning, the suggested mechanism of action of prolactin for the induction of the yawning response needs to be reconsidered.

11. Conclusions

The studies reviewed above show that yawning is under the central control of several neurotransmitters and neuropeptides. Some of the substances interact in the paraventricular nucleus of the hypothalamus to facilitate or inhibit, respectively, the expression of this behavioral response. Very briefly, the available data suggest that oxytocinergic neurons originating in this hypothalamic nucleus and projecting to extra-hypothalamic brain areas (e.g. the hippocampus, the pons and/or the medulla oblongata) mediate the expression of yawning in several circumstances. The activation of these neurons by dopamine, excitatory amino acids and oxytocin itself causes yawning, while their inhibition by, for instance, opioid peptides, prevents the behavioral response. The activation and/or the inhibition of oxytocinergic neurons is apparently mediated by the activation and/or the inhibition of nitric oxide synthase that is present in these neurons in which NO acts as intracellular messenger (Fig. 1).

The paraventricular nucleus and oxytocinergic neurons are apparently not involved in the yawning induced by 5-HT_{2C} receptor agonists or by ACTH–MSH peptides.

Although the possibility cannot be ruled out that the latter compounds act at sites located after the oxytocinergic neurons, there are other neuronal pathways that influence yawning. This also applies to the experimental evidence showing a clear involvement of cholinergic, noradrenergic and GABAergic systems in the expression of yawning. The multiplicity of pathways influencing yawning is well illustrated by the studies showing that there are several links among these neurotransmitters and neuropeptides, e.g. the dopamine–acetylcholine and dopamine–serotonin links (see Sections 3 and 9 for details). However, in this regard, it is pertinent to recall that the interpretation of results of these studies is complicated by the fact that the yawning response is almost always studied after systemic administration of drugs that interact with dopaminergic, serotoninergic and cholinergic transmission. The effects observed thus reflect an overall response to these compounds acting across the entire brain, rather than reveal the involvement of a specific neuronal system. In the case of the dopamine–serotonin link, another complication derives from the fact that many 5-HT receptor agonists and antagonists also interact with dopaminergic receptors, so that it is often very difficult to identify the neuronal system(s) involved in the yawning response induced by these drugs. Consequently, further studies are necessary to clarify whether the observation of interactions between dopamine and serotonin and dopamine and acetylcholine, really reflects direct links between these neuronal systems. Should this be the case, the brain sites of the interactions as well as what causes the activation of these different systems under the various conditions that are characterized by the appearance of yawning need to be demonstrated. Studies with these aims will be also helpful in identifying other brain areas and neuronal systems involved in the control of yawning. There may also result a better understanding of the relationship between these systems and the oxytocinergic system in the modulation of yawning. In view of the presence of oxytocin in several nuclei of the medulla oblongata (i.e. dorsal motor nucleus of the vagus, nucleus ambiguus) (see Argiolas and Gessa, 1991 and references therein) and of the fact that the medulla oblongata contains the neural pathways mediating the well known pattern of the motor inspiratory/expiratory output of yawning (see Introduction), such studies will contribute to the understanding of the neurophysiology and neurochemistry of other responses (i.e. facial mimics, gaping, deglutition and respiration) which also involve central mechanisms of sophisticated neural oromotor control, and may share important features with this phylogenetically old event.

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