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CENTRAL DOPAMINE-OXYTOCIN-ADRENOCORTICOTROPIN LINK IN THE EXPRESSION OF YAWNING AND PENILE ERECTION

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Summary. – *The existence of a neuronal link among dopamine, oxytocin and adrenocorticotropin (ACTH) in the central nervous system is suggested by the ability of these substances to induce both yawning and penile erection in experimental animals. We have performed several experiments in an attempt to clarify this link. 1) We studied the effect of dopamine antagonists, such as haloperidol and sulpiride, on yawning and penile erection induced by apomorphine, oxytocin and ACTH 1-24. Both haloperidol and sulpiride were found able to prevent apomorphine-induced response but not that of oxytocin and ACTH 1-24. 2) The i.c.v. injection of the oxytocin antagonist d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin was found able to antagonize yawning and penile erection induced by oxytocin and apomorphine, but not by ACTH 1-24. 3) The depletion of hypothalamic ACTH and MSH by neonatal monosodium glutamate was found to be ineffective in antagonizing yawning and penile erection either by ACTH 1-24 or oxytocin or apomorphine. 4) Microinjection studies revealed that the paraventricular nucleus of the hypothalamus is the most sensitive brain area for the induction of yawning and penile erection either by apomorphine or oxytocin. Taken together, the present results suggest that DA agonists induce yawning and penile erection by releasing oxytocin in the hypothalamic paraventricular nucleus and that ACTH-derived peptides act either downhill to oxytocin and DA receptors or by a different mechanism to induce such responses.*

Riassunto (Rapporti dopamina-ossitocina - ACTH nello sbadiglio ed erezione peniena). – *L'abilità dei farmaci dopaminomimetici, dell'ossitocina e dell'adrenocorticotropina (ACTH) di indurre sbadiglio ed erezione peniena in diversi animali di laboratorio, suggerisce l'esistenza di un'interazione tra dopamina, ossitocina e ACTH nel sistema nervoso centrale. Per dimostrare e caratterizzare tale interazione sono stati fatti diversi esperimenti i cui risultati possono essere così riassunti: 1) Farmaci dopamino-antagonisti, quali aloperidolo e sulpiride, prevengono lo sbadiglio e l'erezione peniena indotti da apomorfina ma non da ossitocina e ACTH 1-24. 2) L'iniezione intraventricolare dell'antagonista dell'ossitocina, d(CH$_2$)$_5$-Tyr(Me)-Orn^8-vasotocina, previene lo sbadiglio e l'erezione peniena indotta da ossitocina e apomorfina, ma non da ACTH 1-24. 3) La distruzione dei neuroni ipotalamici opiomelanotropinergici mediante somministrazione di monosodio glutamato alla nascita, non ha alcun effetto sullo sbadiglio e sull'erezione peniena indotta da apomorfina, ossitocina e ACTH 1-24. 4) Microiniezione di ossitocina o apomorfina in diverse aree cerebrali indicano che il nucleo paraventricolare dell'ipotalamo è la regione cerebrale più sensibile per l'induzione dello sbadiglio e dell'erezione peniena da parte dell'apomorfina e dell'ossitocina. Tutti insieme i risultati indicano che i farmaci dopaminomimetici inducono sbadiglio ed erezione peniena liberando ossitocina nel nucleo paraventricolare ipotalamico. D'altra parte, i peptidi ACTH-simili sembrano indurre sbadiglio ed erezione peniena o agendo a valle della dopamina e dell'ossitocina o mediante meccanismi differenti.*

Introduction

A peculiar symptomatology characterized by repeated episodes of yawning and penile erection can be induced in experimental animals by the systemic administration of low doses of dopamine (DA) agonists, such as apomorphine (for a review see [1]), by the central administration of adrenocorticotropin (ACTH), α-melanocyte stimulating hormone (α-MSH) and related peptides (for a review see [2]), and by the intracerebroventricular (i.c.v.) injection of oxytocin [3]. While the importance of penile erection in reproduction does not need to be further stressed, it is pertinent to recall that yawning, alone or associated with stretching is considered an ancestral vestige survived during the evolution and that subserves a purpose of arousal. In particular, the role of

yawning would be that of increasing attention when sleep is pressing for fatigue or boredom, but sleep is not allowed as in face of a danger or in social circumstances (for a review on the physiological significance of yawning see [4]).

The capability of the above unrelated substances to induce such similar symptomatology, raises the possibility that a neuronal link exists among DA, oxytocin and ACTH in the central nervous system. The results of the experiments presented below, that were performed to verify the existence of such a link and to clarify the neuronal mechanisms underlying the expression of yawning and penile erection as well, provide evidence that DA, oxytocin and ACTH might act in the hypothalamus in a sequence to induce these behavioural responses.

Material and methods

Male Sprague Dawley rats (250-350 g) (Charles River, Como, Italy) were used in all the experiments. The animals were caged in groups of 4-6 at 22° C with water and standard laboratory food *ad libitum*.

Chronic guide cannulae implantation. – Stainless-steel guide cannulae (22 gauge) aimed at one lateral ventricle (i.c.v.) or different brain areas, were stereotaxically implanted under chlorale hydrate anaesthesia 5 days before the experiments. Coordinates were according to the Pellegrino & Cushman atlas of the rate brain [5]. Chronic guide cannulae, which extended 2 mm below the dura, were implanted bilaterally when aimed at the caudate nucleus (A = 2.6, L = ± 2.8, V = 5.0), nucleus accumbens (A = 3.2, L = ± 1.8, V = 6.5), *substantia nigra* (P = 3.0, L = ± 2.5, V = 8.2), preoptic area (A = 2.0, L = ± 1.5, V = 8.0), ventromedial nucleus (A = 0, L = ± 1.0, V = 8.0), and monolaterally when aimed at the paraventricular nucleus (PVN) (A = 0.2, L = 0.4, V = 7.3), the dorsomedial nucleus (P = 0.2, L = 0.5, V = 8.0), and one of the lateral ventricles (A = 1.0, L = 1.5, V = 5.0).

Microinjections. – Drugs and peptides dissolved in saline or saline alone were injected in a volume of 5 µl into a lateral ventricle or 0.3 µl/2 min for each injected nucleus, by means of an internal cannula (28 gauge) connected by a polyethylene tubing to a 10 µl Hamilton syringe driven by a Stelting microinfusion pump. The lenght of the internal cannula was adjusted according to the position of the injected nucleus. After microinjections, the cannula was left for 30 s in the injection site to allow the spread of the injected solution.

Behavioural studies. – After microinjections, the animals were placed individually into Plexiglas cages (30 × 30 × 25 cm) and observed for 60 min, during which the number of penile erection and yawning epidoses were counted.

Histology. – At the end of the experiments, the animals were killed by decapitation. Brains were rapidly removed and stored in saline containing 2% formaldehyde for 12-15 days. In order to localyze the injection site, 50 µm transverse brain sections were prepared by means of a freezing microtome, stained with Neutral Red and inspected on a phase contrast microscope. Only the animals that were found to have the tip of the cannula positioned correctly, as determined with the aid of a rat brain atlas [5] were considered for the statistical evaluation of the data (Student's t test or Duncan's new multiple range test).

Drugs. – Haloperidol (Janssen, Beerse, Belgium), and (-)sulpiride (Ravizza, Milan, Italy) were dissolved with a drop of concentrated acetic acid, diluted with saline (final pH = 4.5) and injected intraperitoneally in a volume of 1 ml/rat. Apomorphine-HCl (Sigma) was dissolved in saline and injected subcutaneously in the back of the neck in a volume of 0.2 ml/rat.

Peptides. – Oxytocin and ACTH 1-24 were purchased from Peninsula Laboratories (San Carlos, CA, USA). $d(CH_2)_5$ Tyr(Me)- Orn^8-vasotocin was kindly provided by Dr. M. Manning (Toledo University, OH, USA) [6].

Results

Effect of DA antagonists on yawning and penile erection induced by apomorphine, oxytocin and ACTH 1-24. – The first group of experiments was aimed at the identification of possible interactions among DA agonists, oxytocin and ACTH-derived peptides in the induction of yawning and penile erection, namely to clarify if oxytocin and/or ACTH induce the above responses by releasing DA in some brain area or vice versa. Table 1 shows the effect of haloperidol and (-)sulpiride, two specific DA receptor blockers, on yawning and penile erection induced by apomorphine, oxytocin and ACTH 1-24. In agreement with previous studies [1-3] apomorphine (80 µg/kg s.c.), oxytocin (30 ng i.c.v.) and ACTH 1-24 (5 µg i.c.v.) induced repeated episodes of yawning and penile erection. Haloperidol (0.2 mg/kg i.p.) and (-)sulpiride (10 mg/kg i.p.) administered 30 min beforehand completely suppressed apomorphine-induced responses; on the contrary, they were ineffective against yawning and penile erection induced by oxytocin and ACTH 1-24, even at the dose of 2 mg/kg and 50 mg/kg, respectively.

Effect of the oxytocin antagonist $d(CH_2)_5$ Tyr(Me)-Orn^8-vasotocin on yawning and penile erec-

Table 1. – *Effect of the DA receptor blockers haloperidol and (-)sulpiride on yawning and penile erection induced by apomorphine, oxytocin and ACTH 1-24*

Pretreatment	mg/kg	Treatment	Yawns/rat Mean ± S.E.M.	Penile erections/rat Means ± S.E.M.
Saline	—	Saline	2.0 ± 0.5	0.3 ± 0.2
Saline	—	Apomorphine	16.5 ± 1.8 (*a*)	4.0 ± 0.5 (*a*)
Haloperidol	0.2	Apomorphine	3.5 ± 0.6 (*b*)	0.5 ± 0.2 (*b*)
(-)Sulpiride	10	Apomorphine	3.5 ± 0.4 (*b*)	0.6 ± 0.3 (*b*)
Saline	—	Oxytocin	18.0 ± 1.6 (*a*)	3.9 ± 0.6 (*a*)
Haloperidol	2	Oxytocin	15.6 ± 1.0 (*a*)	3.0 ± 0.5 (*a*)
(-)Sulpiride	50	Oxytocin	17.8 ± 2.0 (*a*)	3.6 ± 0.7 (*a*)
Saline	—	ACTH 1-24	16.5 ± 1.3 (*a*)	3.8 ± 0.8 (*a*)
Haloperidol	2	ACTH 1-24	15.6 ± 1.4 (*a*)	3.4 ± 1.0 (*a*)
(-)Sulpiride	50	ACTH 1-24	17.0 ± 1.2 (*a*)	3.5 ± 0.6 (*a*)

Haloperidol and sulpiride were injected intraperitoneally in 1 ml of saline per rat at a pH of 4.5-5. Saline was injected in controls. Apomorphine-HCl was dissolved in saline and injected in a volume of 200 µl in the back of the neck, 30 min after haloperidol and sulpiride. Oxytocin (30 ng) and ACTH 1-24 (5 µg) were dissolved in saline and injected i.c.v. in a volume of 10 µl, 30 min after neuroleptics. The same volume of saline was injected i.c.v. in controls. After treatments, animals were placed individually in Plexiglas cage (30 × 30 × 25 cm) and observed for 60 min during which yawning and penile erection episodes were counted. Each value is the mean ± S.E.M. of 3 experiments (15 rats per group). (*a*) $p < 0.001$ with respect to saline-trated rats; (*b*) $p < 0.001$ with respect to the corresponding group pretreated with saline (Duncan's new multiple range test).

tion induced by apomorphine, oxytocin and ACTH 1-24. – Fig. 1 shows the effect of the i.c.v. injection of the potent oxytocin antagonist d$(CH_2)_5$ Tyr(Me)-Orn^8-vasotocin on yawning and penile erection in-

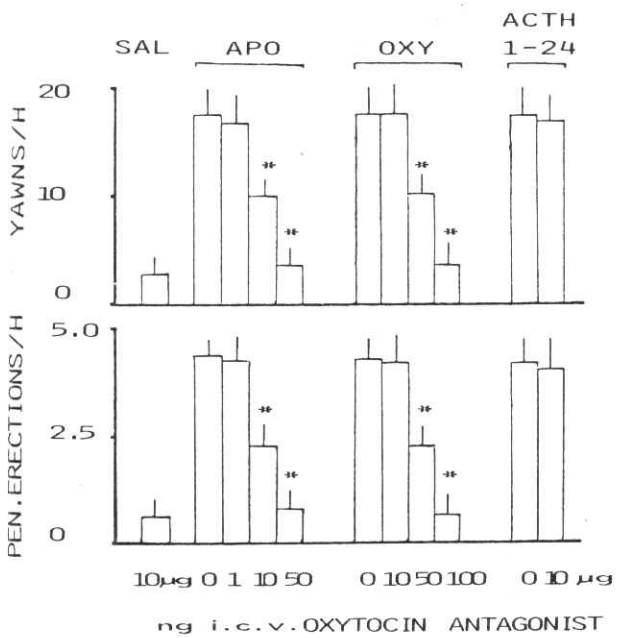

Fig. 1. - Effect of d$(CH_2)_5$-Tyr (Me)-Orn^8-vasotocin on penile erection and yawning induced by apomorphine, oxytocin and ACTH 1-24. I.C.V. pretreatment with saline or d$(CH_2)_5$-Tyr (Me)-Orn^8-vasotocin was performed 15 min before i.c.v. oxytocin (30 ng), ACTH 1-24 (5 µg) or apomorphine (80 µg/kg s.c.). After treatment the animals were placed individually into Plexiglas cages and observed for 60 min during which penile erection and yawning episodes were counted.

* $p < 0.001$ with respect to the corresponding group not receiving d$(CH_2)_5$-Tyr (Me)-Orn^8-VT. (Duncan's new multiple range test).

duced by apomorphine, oxytocin and ACTH 1-24. The oxytocin antagonist injected i.c.v. 15 min before the administration of the above substances, antagonized in a dose-dependent manner not only oxytocin-induced responses, but also, even more effectively, that induced by apomorphine. A 50% inhibition of apomorphine and oxytocin effect was already obtained with 10 ng and 50 ng of the oxytocin antagonist, respectively. A complete suppression of either apomorphine or oxytocin effect was obtained with 100 ng of the peptide. On the contrary, a dose even up to 10 µg of the oxytocin analog was unable to antagonize yawning and penile erection induced by 5 µg of i.c.v. ACTH 1-24. It is noteworthy that doses of the oxytocin antagonist that suppressed apomorphine-induced yawning and penile erection, were totally ineffective in antagonizing stereotypy and hypermotility induced by 1 mg/kg s.c. of the drug (results not shown).

Effect of neonatal monosodium glutamate (MSG) treatment on yawning and penile erection induced by apomorphine, oxytocin and ACTH 1-24. – The results obtained with DA and oxytocin antagonists suggested that DA, oxytocin and ACTH might act in a sequence to induce yawning and penile erection. However, the possibility that DA and/or oxytocin induce the above responses by releasing an ACTH-derived peptide from hypothalamic opiomelanotropinergic neurons (for a review see [7]), remains to be verified. Since specific antagonists of ACTH-MSH peptides capable of antagonizing their central effects are not available at present, we have attempted to verify the above possibility by studying the effect of

apomorphine, oxytocin and ACTH 1-24 on yawning and penile erection in rats neonatally treated with MSG. Such treatment is known to cause the almost complete depletion of brain ACTH-, MSH- and endorphin-like peptides without altering their pituitary and circulating concentrations [8, 9]. Under our conditions (see legend of Table 2) neonatal MSG caused both the expected reduction in growth [10], and a decrease of about 90% in the hypothalamic concentrations of ACTH and α-MSH, as measured by specific radioimmunoassays [11, 12] (not shown). The results obtained with neonatally MSG-treated rats are shown in Table 2. Surprisingly, the depletion of ACTH -MSH-like peptides from the hypothalamus was completely ineffective in modifying yawning and penile erection induced not only by apomorphine and oxytocin, but also by ACTH 1-24.

Oxytocin- and apomorphine-induced yawning and penile erection: site of action in brain. – Beside previous studies showing that ACTH-MSH peptides induce yawning and penile erection by acting into the hypothalamic regions surrounding the third ventricle [2], no information was available so far about the brain areas where DA agonists and/or oxytocin act in order to induce such responses. In an attempt to identify these brain areas, apomorphine and oxytocin microinjections were performed in discrete brain regions through chronic guide cannulae (see Methods). The brain areas that were microinjected with saline, apomorphine (1 µg) and oxytocin (30 ng) are listed in Table 3. The paraventricular nucleus of the hypothalamus (PVN) was found to be the only area where microinjections of apomorphine and oxytocin induced yawning and penile erection. Surprisingly, no effect was observed when apomorphine was injected in areas very rich in DA and DA receptors such as the striatum, the nucleus accumbens or the *substantia nigra*. Ineffective were also microinjections of apomorphine or oxytocin in other hypothalamic nuclei very close to the PVN, such as the ventromedial and dorsomedial nuclei and the preoptic area. The effect of apomorphine and oxytocin microinjections into the PVN was then studied in detail. As shown in Fig. 2, yawning and

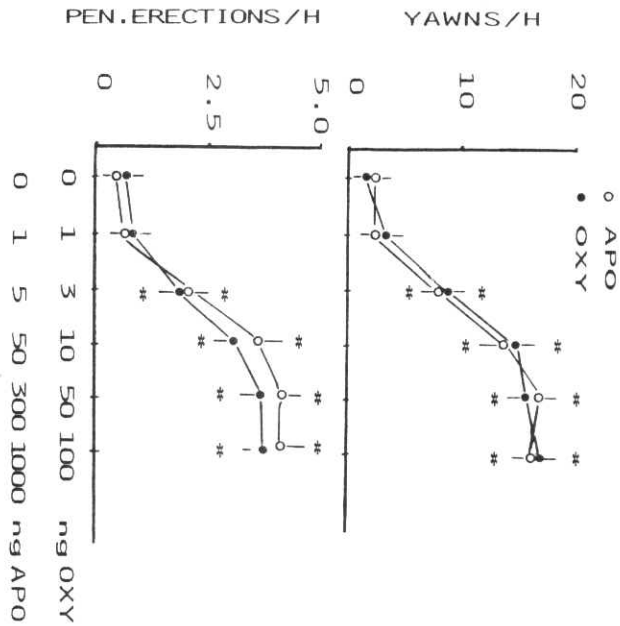

Fig. 2. - Yawning and penile erection induced by apomorphine and oxytocin microinjections into the paraventricular nucleus of the hypothalamus. Apomorphine and oxytocin were microinjected monolaterally into the paraventricular nucleus in a volume of 0.3 µl. The same volume of saline was injected in control rats. Microinjections were done through chronic guide cannulae aimed at the hypothalamic nucleus by means of an internal cannula connected by a polyethylene tubing to a 10 µl Hamilton syringe driven by a Stelting microinfusion pump. After microinjections, the animals were placed individually in Plexiglas cages and observed for 60 min during which penile erection and yawning episodes were counted.

* $p < 0.001$ with respect to saline treated rats (Duncan's multiple range test).

Table 2. – *Yawning and penile erection in monosodium glutamate (MSG)-treated rats; effect of apomorphine, oxytocin and ACTH 1-24*

Treatment	Dose	Neonatal treatment			
		Yawns/rat Mean ± S.E.M.		Penile erections/rat Mean ± S.E.M.	
		Saline	MSG	Saline	MSG
Saline	1 ml i.p.	2.9 ± 0.3	2.0 40.5	0.3 ± 0.2	0.5 ± 0.2
Apomorphine	80 µg/kg s.c.	18.5 ± 1.0 (*a*)	17.2 ± 0.9 (*a*)	3.6 ± 0.5 (*a*)	3.6 ± 0.3 (*a*)
Oxytocin	30 ng i.c.v.	18.0 ± 1.3 (*a*)	18.2 ± 1.8 (*a*)	3.9 ± 0.6 (*a*)	3.5 ± 0.5 (*a*)
ACTH 1-24	1 µg i.c.v.	3.0 ± 0.6	2.7 ± 0.5 (*a*)	0.6 ± 0.1	0.9 ± 0.3
ACTH 1-24	10 µg i.c.v.	19.0 ± 3.0 (*a*)	18.5 ± 2.0 (*a*)	3.9 ± 0.5 (*a*)	3.2 ± 0.4 (*a*)

Neonatal saline and MSG treatment was done in pups in the 1st, 3rd, 5th, 7th and 9th day of life by administering 4 g/kg of MSG or the same volume of saline. The experiments were performed when the rats were 4 months old. At this age, rats were chronically implanted with guide cannulae aimed at one lateral ventricle 5 days before the experiments as described in the test. Other conditions for apomorphine, oxytocin and ACTH 1-24 are the same described in the legend of Table 2 and Fig. 2. Each value is the mean ± S.E.M. of 2 experiments (10 rats per group). Yawning and penile erection episodes were counted for 60 min after treatment. (*a*) $p < 0.001$ with respect to saline-treated rats (Duncan's new multiple range test).

Table 3. – *Yawning and penile erection by apomorphine and oxytocin microinjections in different brain areas*

Brain Area	Effect of microinjections of:	
	Apomorphine	Oxytocin
Striatum	None	None
Nucleus accumbens	None	None
Substantia nigra	None	None
Paraventricular nucleus	Yawning, pen. erection	Yawning, pen. erection
Dorsomedial nucleus	None	None
Ventromedial nucleus	None	None
Preoptic area	None	None

One µg of apomorphine or 10 ng of oxytocin were injected in each site through bilateral chronic guide cannulae by means of an internal cannula connected by a polyethylene tubing to a 10 µl Hamilton syringe driven by a Stelting microinfusion pump, except for the paraventricular and dorsomedial nucleus where 1 µg of apomorphine and 10 ng of oxytocin were injected monolaterally. The injection volume was 0.3 µl per site. After microinjections, rats were placed individually in Plexiglas cages and observed for 60 min during which yawning and penile erection episodes were counted. Ten rats per group were used: 4 were injected with saline alone (controls), and the other 6 with apomorphine or oxytocin. The correct position of the cannula tip into the various nuclei was verified by histological analysis.

penile erection were induced in a dose-dependent manner by both substances. The minimal effective dose of oxytocin and apomorphine was 3 and 5 ng, respectively, that induced the response in about 60% of the treated animals. The symptomatology after apomorphine or oxytocin microinjection into the PVN was similar to that observed after systemic apomorphine or i.c.v. oxytocin, with the only difference that response started within 5 min after the microinjections. Even at the highest dose tested (1 µg), apomorphine failed to induce stereotypy and hypermotility.

Discussion

The present results show that DA agonists induce yawning and penile erection by releasing oxytocin in the central nervous system. This hypothesis is supported by: 1) the failure of neuroleptics to antagonize oxytocin-induced responses, and 2) the potency of the oxytocin antagonist $d(CH_2)_5$ Tyr(Me)-Orn^8-vasotocin to prevent either apomorphine or oxytocin effects. Moreover, the microinjection studies suggest that the brain area where DA agonists apparently act to induce oxytocin release is the hypothalamic PVN. Indeed, the potency of the two substances together with the fact that both DA and oxytocin are present in this nucleus, suggests that DA and oxytocin might have a physiological role in the control of the above responses. Accordingly, the PVN contains the cell bodies of at least two types of oxytocinergic neurons: the magnocellular neurons, projecting to the neurohypophysis from which oxytocin is released in the circulation to exert its hormonal role in parturition and lactation (for a review see [13]), and the parvocellular neurons, many of which send their projections to several extrahypothalamic brain areas [14, 15]. In addition to oxytocinergic cell bodies, the PVN contains also the cell bodies of dopaminergic neurons of the group A14 [16], that together with those of the groups A11 and A13, constitute the so-called incertohypothalamic DA system [17]. The finding suggests a direct involvement of this DA system in the expression of yawning and penile erection. In particular, our results suggest that Da agonists interact with DA receptors in the PVN or surrounding structures to stimulate the activity of oxytocinergic neurons, which in turn mediate the appearance of yawning and penile erection. In support of this hypothesis, immunocytochemical studies have shown that DA neurons in the PVN are mainly located in the proximity of oxytocinergic neurons [18]. As to the kind of DA receptors mediating yawning and penile erection, previous studies have shown that they belong to the D2 type, although it is still controversial if they are DA autoreceptors (a special kind of DA receptors located in the nervous terminal and cell body of the neuron itself) or postsynaptic DA receptors (for a review on this subject see [19]).

As to the mechanism by which oxytocin acts in the PVN to induce yawning and penile erection, only some speculation is possible at present. A possible explanation is that oxytocin activates oxytocinergic neurons. According to this hypothesis, oxytocinergic receptors have been identified in the rat PVN [20], and exogenous oxytocin has been found to increase *in vivo* the activity of oxytocinergic neurons [21] and to stimulate *in vitro* the release of endogenous oxytocin [22]. Furthermore, oxytocinergic synapses have been found to impinge on oxytocinergic neurons in hypothalamic nuclei [23].

On the other hand, the failure of neuroleptics and oxytocin antagonist to prevent ACTH-induced yawning and penile erection, together with the inability of the hypothalamic depletion of ACTH-derived peptides by MSG treatment to modify the apomorphine-, oxytocin- and ACTH-induced responses suggests that: 1) DA agonists and oxytocin do not induce yawning and penile erection by releasing an ACTH-derived peptide from hypothalamic opiomelanotropinergic neurons, although the possibility that the remaining ACTH can be still enough to mediate apomorphine and/or oxytocin effect cannot be completely ruled out, and 2) ACTH-MSH peptides induce their effect on yawning and penile erection by acting at sites localized in the hypothalamus, but situated either downhill to DA and oxytocin receptors or by a different mechanism not involving hypothalamic DA or oxytocin.

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REFERENCES

1. SERRA, G., COLLU, M., GESSA, G.L. & FERRARI, W. 1986. Melanocortins and dopamine link in yawning behaviour. In: *Central actions of ACTH and related peptides*. D. De Wied & W. Ferrari (Eds). *Symposia in Neuroscience*. Liviana Press, Padua, Italy. **4**: 163-178.

2. BERTOLINI, A. & GESSA, G.L. 1981. Behavioural effects of ACTH-MSH peptides. *J. Endocrinol. Inv.* **4**: 241-251.

3. ARGIOLAS, A., MELIS, M.R. & GESSA, G.L. 1986. Oxytocin, an extremely potent inducer of penile erection and yawning. *Eur. J. Pharmacol.* **130**: 265-272.

4. FERRARI, W., GESSA, G.L. & VARGIU, L. 1963. Behavioural effects induced by intracisternally injected ACTH and MSH. *Ann. NY Acad. Sci.* **104**: 330-345.

5. PELLEGRINO, L.J. & CUSHMAN, A.J. 1971. *A stereotaxic atlas of the rat brain*. Meredith, New York.

6. BANKOWSKI, K., MANNING, M., SETO, J., HALDER, J. & SAWYER, W.H. 1980. Design and synthesis of potent *in vivo* antagonists of oxytocin. *Int. J. Protein Res.* **16**: 382-391.

7. O'DONOHUE, T.L. & DORSA, D.M. 1982. The opiomelanotropinergic neuronal and endocrine system. *Peptides* **3**: 353-395.

8. ESKAY, R.L., BROWNSTEIN, M.J. & LONG, R.T. 1979. Melanocyte stimulating hormone: reduction in adult brain after monosodium glutamate treatment in neonats. *Science* **205**: 827-828.

9. KRIEGER, D.T., LIOTTA, A.S., NICHOLSON, G. & KIZER, J.S. 1979. Brain ACTH and endorphin reduced in rats with monosodium glutamate-induced arcuate nuclear lesions. *Nature* **278**: 562-563.

10. BLOCH, B., LING, N., BENOIT, R., WEHRENBERG, W.B. & GUILLEMIN, R. 1984. Specific depletion of immunoreactive growth hormone-releasing factor by monosodium glutamate in rat median eminence. *Nature* **307**: 272-273.

11. ARGIOLAS, A., MELIS, M.R., FRATTA, W. & GESSA, G.L. 1986. Existence of different forms of adrenocorticotropin in rat hypothalamus. *Peptides* **7**: 591.596.

12. FRATTA, W., ARGIOLAS, A., FADDA, F., GESSA, G.L. & FERRARI, W. 1986. Involvement of ACTH-MSH peptides in opiate withdrawal syndrome. In: *Central actions of ACTH and related peptides*. D. De Wied & W. Ferrari (Eds). *Symposia in Neuroscience*. Liviana Press, Padua, Italy. **4**: 223-230.

13. POULAIN, D.A. & WAKERLY, J.B. 1982. Electrophysiology of hypothalamic neurons secreting oxytocin and vasopressin. *Neuroscience* **7**: 773-808.

14. BUIJS, R.M. 1978. Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *Cell Tissue Res.* **192**: 423-435.

15. SOFRONIEW, M.W. 1983. Vasopressin and oxytocin in the mammalian brain and spinal cord. *Trends Neurosci.* **6**: 467-472.

16. DAHLSTROM, A. & FUXE, K. 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I: Demonstration of monoamines in the cell bodies of brainstem neurones. *Acta Physiol. Scand.* **62** (Suppl. 232): 1-55.

17. LINDVALL, O. & BJORKLUND, A. 1978. Anatomy of the dopaminergic neurons systems in the rat brain. Raven Press, New York. *Adv. Biochem. Pshychopharmacol.* **19**: 1-23.

18. SWANSSON, L.W. & SAWCHENKO, P.E. 1983. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Ann. Rev. Neurosci.* **6**: 269-324.

19. SERRA, G., COLLU, M. & GESSA, G.L. 1986. Dopamine receptors mediating yawning: are they autoreceptors? *Eur. J. Pharmacol.* **120**: 187-192.

20. BRINTON, R.E., WAMSLEY, J.K., GEE, K.W., WAN YEIH, P. & YAMAMURA, H.I. 1984. ^{3}H-oxytocin binding sites in the rat brain demonstrated by quantitative light microscopic autoradiography. *Eur. J. Pharmacol.* **102**: 365-367.

21. FREUND-MERCIER, M.J. & RICHARD, P. 1981. Excitatory effects of intraventricular injections of oxytocin on the milk ejection reflex in the rat. *Neurosci. Lett.* **23**: 193-198.

22. MOOS, F., MERCIER, M.J., GUERNE', Y., STOECKEL, M.E. & RICHARD, P. 1984. Release of oxytocin and vasopressin by magnocellular nuclei *in vitro*: specific facilitatory effect of oxytocin on its own release. *J. Endocrinol.* **102**: 63-72.

23. THEODOSIS, D.T. 1985. Oxytocin-immunoreactive terminals synapse on oxytocinergic neurones in the supraoptic nuclei. *Nature* **313**: 682-684.