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ω-Conotoxin Prevents Apomorphine- and Oxytocin-Induced Penile Erection and Yawning in Male Rats

A. ARGIOLAS, M. R. MELIS, R. STANCAMPIANO AND G. L. GESSA

B. B. Brodie Department of Neurosciences, University of Cagliari
Via Porcell 4, 09124 Cagliari, Italy

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ARGIOLAS, A., M. R. MELIS, R. STANCAMPIANO AND G. L. GESSA. *ω-Conotoxin prevents apomorphine- and oxytocin-induced penile erection and yawning in male rats.* PHARMACOL BIOCHEM BEHAV **37**(2) 253–257, 1990.—The effect of the intracerebroventricular (ICV) administration of ω-conotoxin GVIA on penile erection and yawning induced by oxytocin or by the dopaminergic agonist apomorphine was studied in male rats. The peptide toxin, 1–10 ng given ICV 5 min before oxytocin (30 ng ICV) or apomorphine (80 μg/kg SC), but not its carboxymethylated (CM) derivative, prevented the above behavioral responses in a dose-dependent manner. Similarly, ω-conotoxin (5 ng) unilaterally injected in the paraventricular nucleus of the hypothalamus (PVN) prevented penile erection and yawning induced by the microinjection of oxytocin (10 ng) or apomorphine (50 ng) in the PVN. ω-Conotoxin injected in the PVN, but not in the preoptic area, prevented also penile erection and yawning induced by systemic apomorphine (80 μg/kg SC). ICV ω-conotoxin was unable to prevent stereotypy induced by apomorphine (500 μg/kg SC). The present results provide further evidence that calcium plays a major role in the expression of penile erection and yawning and that apomorphine and oxytocin induce these behavioral responses by mobilizing calcium through ω-conotoxin-sensitive (N-type) calcium channels.

Oxytocin Apomorphine Penile erection Yawning ω-Conotoxin Calcium channels Rat

REPEATED episodes of penile erection and yawning can be induced in rats either by oxytocin [see (1)] or by dopaminergic agonists such as apomorphine, which is believed to induce these effects by releasing oxytocin in the central nervous system [see (19,20)]. This symptomatology is prevented by organic calcium channel inhibitors in a dose-dependent manner (3,5), suggesting that calcium plays a major role in the expression of these behavioral responses. However, the possibility that the prevention of apomorphine and oxytocin effect by calcium channel inhibitors might be due to unspecific effects of these compounds cannot be completely ruled out because of the relatively high doses required to prevent the above behavioral responses. In fact, brain neuronal tissue is poorly sensitive to these compounds [for a review see (12)] due to a subtype of calcium channel (N-type) different from those found in cardiac and smooth muscle tissue (16,28). Recently, ω-conotoxin GVIA, a 27-residue peptide toxin isolated from the venom of the fish-hunting snail *Conus geographus* (24,25), which has been found to be a rather selective inhibitor of N-type calcium channels (16,28), has become commercially available. This prompted us to investigate the effect of this peptide either after injection in a lateral ventricle (ICV) or in the paraventricular nucleus of the hypothalamus (PVN), one of the most sensitive brain areas for the induction of penile erection and yawning by apomorphine and oxytocin, on the expression of these behavioral responses.

METHOD

Male Sprague-Dawley rats (200–250 g, Morini, Bologna, Italy) were used in all the experiments. Rats were caged in groups of 4–6 at 24°C, humidity 60%, with water and standard laboratory food ad lib.

ICV, PVN and Preoptic Area (POA) Injections

Stainless steel guide cannulas (22 gauge) aimed at one lateral ventricle or unilaterally at the PVN or bilaterally at the POA were stereotaxically implanted (David Kopf Instruments, USA) under chloral hydrate anaesthesia 5 days before the experiments (coordinates: lateral ventricle, 1 mm anterior to bregma, 1.5 mm lateral to midline and 22 mm ventral to dura; PVN, 0.2 mm anterior to bregma, 0.4 mm lateral to midline and 2 mm ventral to dura; POA, 2.0 mm anterior to bregma, 2 mm lateral to midline and 2 mm ventral to dura) (26). Peptides dissolved in saline or saline alone were injected ICV via an internal cannula (28 gauge), which extended 2 mm below the tip of the guide cannula and connected by polyethylene tubing to a 10 μl Hamilton syringe driven by a micrometric screw. For PVN and POA microinjections, peptides dissolved in saline or saline alone (0.3 μl in 2 min) were injected unilaterally in the PVN and bilaterally in the POA by means of an internal cannula (28 gauge) which extended 5.3 and 6.0 mm, respectively, below the tip of the guide cannula and connected to a 10 μl Hamilton syringe driven by a Stoelting microinfusion pump. After injection the tip of the cannula was left in the injection site for 30 sec to allow the spread of the injected solution.

Systemic Treatments

Apomorphine-HCl (Sigma) was dissolved in saline and in-

jected subcutaneously (SC) in the back of the neck in a volume of 0.1 ml/100 g body weight. Controls received the same volume of saline alone.

Peptides

Synthetic oxytocin and ω-conotoxin GVIA were purchased from Peninsula Laboratories (Palo Alto, CA). Carboxymethylated (CM)-ω-conotoxin was prepared from ω-conotoxin after reduction of the disulfide bridges with dithiothreitol and carboxymethylation with iodoacetic acid according to classical procedures, and purified by high pressure liquid chromatography on a 0.39 × 30 cm μBondapak C18 column (Waters Associates). The chromatographic conditions were reported elsewhere (1) except that the gradient was linear from 5% to 30% Solv.B in 30 min. Under these conditions CM-ω-conotoxin was well separated from intact ω-conotoxin, as indicated by their retention time.

Behavioral Studies

In the experiments in which ω-conotoxin or CM-ω-conotoxin were given ICV, ICV oxytocin or SC apomorphine were administered 5 min after pretreatment either with peptide or with vehicle. Soon after treatment, the animals were placed individually into Plexiglas cages (30 × 30 × 30 cm) and observed for 60 or 45 min after oxytocin or apomorphine, respectively, during which penile erection and yawning episodes were counted. At the end of the experiments, the animals were killed by decapitation, the brains were removed and visually inspected to ascertain the correct position of the cannula tip into the lateral ventricle. In the experiments in which ω-conotoxin was microinjected in the PVN, oxytocin or apomorphine were also microinjected in the PVN 5 min after the peptide toxin. When ω-conotoxin was microinjected in the POA, apomorphine was given SC 5 min later. At the end of the experiment rats were killed by decapitation, the brains removed and stored in saline containing 2% formaldehyde for 12–15 days. In order to localize 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 those animals that were found to have the cannula tip positioned correctly in the lateral ventricle or in the PVN or in the POA were considered for statistical analysis of the results (Duncan's multiple range test). In those animals which were treated with high doses of apomorphine, stereotypy was quantified by measuring the % of time spent by the rats in locomotion, sniffing and gnawing during the observation period.

RESULTS

Effect of ICV ω-Conotoxin

As shown in Fig. 1, penile erection and yawning induced by oxytocin (30 ng ICV) or by apomorphine (80 μg/kg SC) in male rats was prevented in a dose-dependent manner by ω-conotoxin, but not CM-ω-conotoxin, given ICV at doses between 1 and 10 ng (≈0.3–3 pmol) 5 min before oxytocin or apomorphine. An almost complete prevention of oxytocin and apomorphine effect was induced by 10 ng of ω-conotoxin. CM-ω-conotoxin was found to be inactive even at the dose of 1 μg (not shown). At the above doses both ω-conotoxin and CM-ω-conotoxin failed to produce any overt behavioral change. In contrast, doses of ω-conotoxin of 100 ng or higher, but not of CM-ω-conotoxin, induced shaking which increased in a dose-dependent manner in all treated rats as already reported in the mouse (24,25). Rats which received 0.5 μg ICV of the toxin showed shaking for at least 2 days, and 8 out of 10 rats which received 1 μg died within 12 hr after treatment

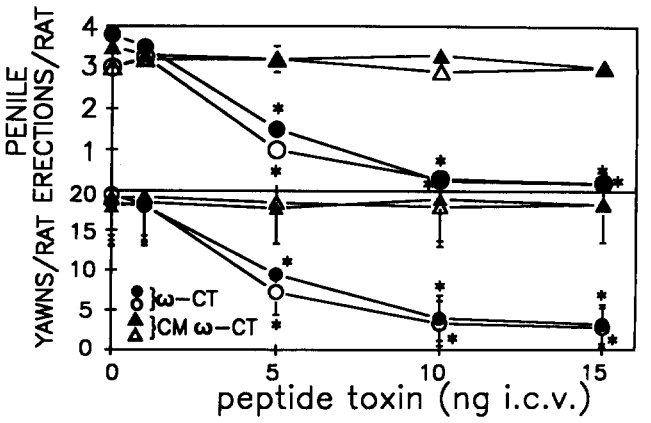

FIG. 1. Prevention of apomorphine- and oxytocin-induced penile erection and yawning by ICV ω-conotoxin GVIA in male rats. Rats received 0, 5, 10 and 15 ng of ICV ω-conotoxin or CM-ω-conotoxin 5 min before oxytocin (30 ng ICV) (open symbols) or apomorphine (80 μg/kg SC) (closed symbols). After oxytocin or apomorphine treatment rats were placed individually in Plexiglas cages and observed for 60 min or 45 min, respectively, during which penile erection and yawning episodes were counted. Values are means ± S.E.M. of 12 rats per each group. *$p<0.001$ with respect to control values (peptide toxin = 0).

(results not shown), in contrast to mice which survived even after the ICV injection of 2 nmole (≈6 μg) which induced shaking for 5 days (25).

Effect of PVN and POA ω-Conotoxin Microinjection

Prevention of oxytocin and apomorphine effects was also found when ω-conotoxin was microinjected in the PVN, the most sensitive brain area for the induction of penile erection and yawning by oxytocin or apomorphine (18,19). Indeed, as shown in Fig. 2, 5 ng of ω-conotoxin, injected unilaterally in the PVN 5 min before oxytocin (10 ng in the PVN) or apomorphine (50 ng in the PVN), prevented oxytocin- or apomorphine-induced penile erection and yawning. ω-Conotoxin microinjected unilaterally in the PVN, but not bilaterally (5 ng per site) in the POA, prevented also penile erection and yawning induced by systemic apomorphine (80 μg/kg SC) (Fig. 3).

Effect of ω-Conotoxin on Stereotypy

In spite of its ability to prevent apomorphine-induced penile erection and yawning, ω-conotoxin (10 ng ICV) was unable to modify stereotyped behaviors (sniffing and gnawing) induced by the dopaminergic agonist (500 μg/kg SC) (Fig. 4).

DISCUSSION

The present results show that nanogram amounts of ICV ω-conotoxin, a potent and selective inhibitor of the N-type calcium channels present in the nervous tissues, including brain neurons (16, 17, 28), prevent oxytocin- and apomorphine-induced penile erection and yawning. The finding is in line with previous results showing that calcium plays a major role in the expression of the above behavioral responses. Accordingly, organic calcium channel inhibitors belonging to different chemical classes (i.e., verapamil, nimodipine, nifedipine, nicadipine and flunarizine) prevent oxytocin- and apomorphine-induced penile erection and yawning in a dose-dependent manner after systemic or ICV administration

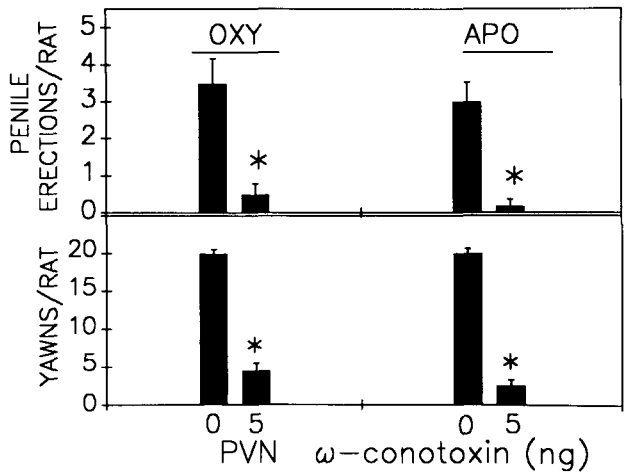

FIG. 2. Prevention of apomorphine- and oxytocin-induced penile erection and yawning by the microinjection of ω-conotoxin GVIA in the PVN of male rats. ω-Conotoxin (5 ng/0.3 μl) was microinjected unilaterally in the PVN through chronic guide cannulas as described in the Method section. Apomorphine (50 ng/0.3 μl) or oxytocin (10 ng/0.3 μl) was microinjected unilaterally in the PVN 5 min after ω-conotoxin. After oxytocin or apomorphine treatment rats were placed individually in Plexiglas cages and observed for 60 min or 45 min, respectively, during which penile erection and yawning episodes were counted. Values are means ± S.E.M. of 10 rats per each group. *$p<0.001$ with respect to control values (peptide toxin = 0).

(3,5). However, in the above studies, the possibility that the prevention of the above responses was due to unspecific effects of calcium channel inhibitors rather than to the blockade of calcium channels could not be ruled out because of the requirement of

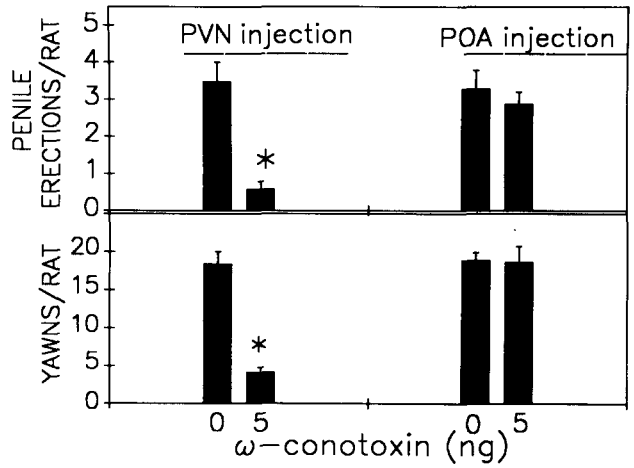

FIG. 3. Prevention of systemic apomorphine-induced penile erection and yawning by the microinjection of ω-conotoxin in the PVN, but not in the POA. ω-Conotoxin (5 ng/0.3 μl) was microinjected unilaterally in the PVN or bilaterally in the POA (5 ng/0.3 μl/site) through chronic guide cannulas as described in the Method section. Apomorphine (80 μg/kg SC) was given 5 min after PVN or POA ω-conotoxin. After apomorphine treatment rats were placed individually in Plexiglas cages and observed for 45 min, during which penile erection and yawning episodes were counted. Values are means ± S.E.M. of 10 rats per each group. *$p<0.001$ with respect to control values (peptide toxin = 0).

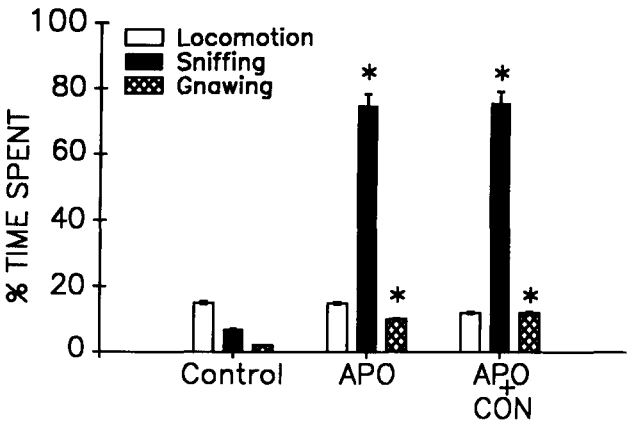

FIG. 4. Failure of ω-conotoxin to modify stereotyped behaviors (sniffing and gnawing) induced by systemic apomorphine. ω-Conotoxin (10 ng ICV) was given 5 min before apomorphine (500 μg/kg SC) or 0.2 ml of saline alone (controls). After treatment rats were placed individually in a Plexiglas cage and the % of time of the observation period (45 min) spent in locomotion, sniffing and gnawing was measured. Values are means ± S.E.M. of 12 rats per group. *$p<0.001$ with respect to control values.

relatively high doses of these compounds. In view of the potency of ω-conotoxin in preventing penile erection and yawning, it is likely that the requirement of high doses of organic calcium channel inhibitors was due to the poor sensitivity of N-type calcium channels to these compounds unlike those of the L- and T-types present in cardiac and muscle tissue [see (12, 16, 28)].

Most important, ω-conotoxin prevents PVN oxytocin- and apomorphine-induced penile erection and yawning also when microinjected unilaterally in the hypothalamic PVN, the most sensitive brain area for the induction of these behavioral responses induced by the above substances (18,19). The finding is in agreement with previous studies showing that the hypothalamic PVN plays a key role in the expression of penile erection and yawning induced by dopaminergic agonists and oxytocin. In this regard, it is pertinent to recall that the PVN contains 1) the cell bodies of at least two kinds of oxytocinergic neurons: the magnocellular neurons that send their projections mainly to the neurohypophysis [see (27)], and the parvocellular neurons, many of which send their projections to extrahypothalamic brain areas (7, 14, 29); and 2) the cell bodies of dopaminergic neurons of the A14 group that constitute, together with those of A11 and A13 groups, the so-called incertohypothalamic dopaminergic system (8,15). Furthermore, both dopamine (11) and oxytocin receptors (6) have been identified in this nucleus, and bilateral electrolytic lesion of the PVN, which causes an almost complete depletion of oxytocinergic neurons within the brain and spinal cord (13,14), abolishes penile erection and yawning induced by both apomorphine and oxytocin (2). The ability of unilateral PVN ω-conotoxin in preventing penile erection and yawning induced by systemic apomorphine is also in line with the findings recalled above, but must be considered with some caution. In fact, although the most likely explanation for this finding is that ω-conotoxin reaches the controlateral PVN, some diffusion of the peptide through the third ventricle into other brain areas as well cannot be ruled out, making it uncertain whether ω-conotoxin acts only in the PVN to prevent the effect of systemic apomorphine.

The prevention by ω-conotoxin of oxytocin- and apomorphine-induced penile erection and yawning provides further support to the hypothesis that a neuronal dopamine-oxytocin link is involved

in the expression of these behavioral responses [see (20)] and that the PVN is the site in which the dopamine-oxytocin interaction takes place. Moreover, taken together with the finding that calcium microinjections in this nucleus induce a symptomatology similar to that induced by oxytocin and apomorphine (5), the present results suggest that calcium plays a major role in this interaction. However, the mechanism by which ω-conotoxin acts in the PVN to prevent oxytocin- and apomorphine-induced penile erection and yawning is unknown, and only some speculation is possible at present. If one assumes that apomorphine induces its effect by acting in the PVN to increase oxytocinergic transmission, as suggested by the ability of oxytocin antagonists to prevent apomorphine-induced penile erection and yawning with a potency parallel to that found in blocking oxytocin receptors (20), one can speculate that ω-conotoxin prevention of apomorphine effect is secondary to the inhibition of oxytocin effect which in turn is mediated by an increase of calcium influx through ω-conotoxin-sensitive calcium channels in the PVN. In agreement with this hypothesis, systemic apomorphine increases oxytocin concentration in male rat plasma (21,31) and also in extrahypothalamic brain areas (22). Calcium channels activated by the stimulation of oxytocinergic receptors and responsible for oxytocin effect might be located on the cell bodies of oxytocinergic neurons projecting to extrahypothalamic brain areas (7, 14, 29), or on oxytocinergic synapses impinging on oxytocinergic cell bodies as well (30). Accordingly, exogenous oxytocin was found to stimulate its own neurons in vivo (9) and to stimulate its own release in vitro (23). Alternatively, these ω-conotoxin-sensitive calcium channels might be located in other neurons involved in the expression of the above behavioral responses whose activity is under oxytocinergic control. However, the present results do not rule out the possibility that apomorphine itself is releasing ocytocin by increasing calcium influx through ω-conotoxin-sensitive calcium channels. These calcium channels might be located either on the cell bodies of the oxytocinergic neurons or on neurons which in turn activate oxytocinergic neurons. Further studies are necessary to clarify this possibility.

Finally, our results suggest that oxytocin induces penile erection and yawning by acting with a mechanism similar to that operating in the uterine or mammary tissue. Accordingly, oxytocin action on these peripheral tissues is dependent on the presence of intra- and extracellular calcium and calcium channel inhibitors prevent oxytocin-induced uterine contractions [for a review, see (10)]. This correlates well with our recent finding showing that uterine-type oxytocin receptors might be responsible for the induction of penile erection and yawning by oxytocin (4). Although further studies are necessary to clarify the mechanism by which oxytocin and/or apomorphine induce calcium mobilization, the present results suggest that ω-conotoxin-sensitive calcium channels located in the hypothalamic PVN play a key role in the expression of penile erection and yawning induced by these compounds and that calcium is the second messenger that mediates these behavioral responses.

ACKNOWLEDGEMENT

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