Spinal proerectile effect of oxytocin in anesthetized rats

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Spinal proerectile effect of oxytocin in anesthetized rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1870–R1877, 2001.—The spinal cord contains the neural network that controls penile erection. This network is activated by information from peripheral and supraspinal origin. We tested the hypothesis that oxytocin (OT), released at the lumbosacral spinal cord level by descending projections from the paraventricular nucleus, regulated penile erection. In anesthetized male rats, blood pressure and intracavernous pressure (ICP) were monitored. Intrathecal (it) injection of cumulative doses of OT and the selective OT agonist [Thr4,Gly7]OT at the lumbosacral level elicited ICP rises whose number, amplitude, and area were dose dependent. Thirty nanograms of OT and one-hundred nanograms of the agonist displayed the greatest proerectile effects. Single injections of OT also elicited ICP rises. Preliminary injection of a specific OT-receptor antagonist, hexamethonium, or bilateral pelvic nerve section impaired the effects of OT injected it. NaCl and vasopressin injected it at the lumbosacral level and OT injected it at the thoracolumbar level or intravenously had no effect on ICP. The results demonstrate that OT, acting at the lumbosacral spinal cord, elicits ICP rises in anesthetized rats. They suggest that OT, released on physiological activation of the PVN in a sexually relevant context, is a potent activator of spinal proerectile neurons.

PHARMACOLOGICAL STIMULATION of the paraventricular nucleus of the hypothalamus (PVN), through a variety of neuroactive compounds in conscious rats and electrical or pharmacological stimulation of the PVN in anesthetized rats, elicits penile erection and intracavernous pressure (ICP) increases (3, 8). The PVN contributes descending oxytocinergic fibers to the spinal cord (7), and the paraventriculospinal tract originates in the parvocellular part of the PVN (15). In male rats, the lumbosacral spinal cord contains oxytocinergic fibers (29), some of which synapse onto spinal preganglionic neurons (30). Furthermore, specific oxytocin (OT) binding sites are present in the sacral parasympathetic nucleus and the dorsal grey commissure of the L6-S1 spinal cord (34). Finally, PVN neurons are transsynaptically labeled with pseudorabies virus (PRV) injected in the corpus cavernosum (18). Engorgement of the penis with blood, leading to erection, is caused by increased blood flow to the penis and active relaxation of the erectile tissue of the corpora cavernosa and the corpus spongiosum (1). Both mechanisms are controlled by the autonomic nervous system. In rats, the sympathetic outflow to the penis originates in the T12-L2 spinal cord, and the proerectile parasympathetic outflow originates in the L6-S1 spinal cord (9).

The sacral parasympathetic nucleus (SPN) of the L6-S1 spinal cord contains the preganglionic neurons that innervate the pelvic organs, including the penis. In a sexually relevant context, activation of proerectile neurons in the SPN may be elicited by information from peripheral or supraspinal origins (24). We tested the hypothesis that OT released by paraventriculospinal pathways could regulate the spinal control of penile erection through an effect on lumbosacral neurons in the rat.

MATERIAL AND METHODS

Animals. Adult male Sprague-Dawley rats, sexually naive and weighing 200–250 g, were purchased from Charles River (Saint-Aubin les Elbeufs, France). Rats were housed in groups of four in plastic cages containing wood-chip bedding. They had free access to commercial pelleted rodent chow (Pietrement, Provins, France) and tap water. Cages were placed in an animal facility maintained at 20°C and kept in a 12:12-h light-dark cycle (lights on at 8 AM). All animal experiments were carried out in accordance with the European Economical Community Directive of November 24, 1986 (86/609/EEC) on the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental procedure. Rats were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg in sterile water), and their temperature was maintained at 37°C using a...
homeothermic blanket. Intrathecal (it) catheterization was performed as reported by LoPachin et al. (17). Briefly, the rat’s head was placed in a stereotaxic frame and was rotated nose downwards to facilitate catheter insertion. The catheter, a polyethylene tubing (PE-10) stretched to 150% of its original length in hot water, was cut to the required length so that its distal opening reached the L4–L6 or T12-T13 levels of the spinal cord. The skin and neck muscles were incised and retracted. The atlantooccipital membrane was opened, and the catheter, flushed with sterile NaCl 0.9%, was carefully advanced in the caudal direction. Finally, the rostral free end of the catheter was secured with the ligatures that closed the neck muscles and skin layers. The catheter was connected to a Hamilton syringe filled with saline to prevent cerebrospinal fluid leakage. Rats were tracheotomized to prevent aspiration of saliva and to perform artificial ventilation when muscle relaxant was used. The carotid artery and jugular vein were catheterized with polyethylene tubings filled with heparinized saline (25 U/ml) to record blood pressure (BP) via a pressure transducer (Elcomatic 750, Glasgow, UK) and inject drugs intravenously, respectively. ICP recording was performed as described previously (12). Tables 1 and 3 display the different groups of rats that we used. For it injections, compounds dissolved in 10 μl of NaCl 0.9% were injected within 10–20 s, immediately followed by a flush of 10 μl NaCl 0.9%. When we used cumulative injections of drugs, two consecutive injections were separated by a 15-min period. To perform pelvic nerve section (PNx rats), a suprapubic incision was performed. The pelvic nerves were exposed at the level of the pelvic nerve; iv, intravenously. *Before cumulative doses of OT intrathecally (it) delivered at the L4–L6 level.

Effects of drugs, injected either it or iv, on intracavernous pressure of anesthetized male rats

Table 1. Effects of drugs, injected either it or iv, on intracavernous pressure of anesthetized male rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route/Site</th>
<th>Dose</th>
<th>Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (0.9%)</td>
<td>it L4–L6</td>
<td>8 injections</td>
<td>10</td>
</tr>
<tr>
<td>OT</td>
<td>it L4–L6</td>
<td>0.3, 1, 3, 10, 30, 100, and 300 ng</td>
<td>10</td>
</tr>
<tr>
<td>OT</td>
<td>it T12–T13</td>
<td>0.3, 1, 3, 10, 30, 100, and 300 ng</td>
<td>10</td>
</tr>
<tr>
<td>OT</td>
<td>iv</td>
<td>0.3, 1, 3, 10, 30, 100, and 300 ng</td>
<td>8</td>
</tr>
<tr>
<td>[Thr⁴, Gly⁷]OT</td>
<td>it L4–L6</td>
<td>0.3, 1, 3, 10, 30, 100, and 300 ng</td>
<td>8</td>
</tr>
<tr>
<td>AVP</td>
<td>it L4–L6</td>
<td>100 ng*</td>
<td>8</td>
</tr>
<tr>
<td>[(S)PMP₁, D-Trp₂, Pen⁶, Arg⁸]OT</td>
<td>it L4–L6</td>
<td>100 ng*</td>
<td>8</td>
</tr>
<tr>
<td>PNX</td>
<td>iv</td>
<td>60 mg/kg*</td>
<td>5</td>
</tr>
<tr>
<td>HMX</td>
<td>iv</td>
<td>30 mg/kg*</td>
<td>5</td>
</tr>
<tr>
<td>Gallamine triethiodide</td>
<td>iv</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NaCl (0.9%), sodium chloride; OT, oxytocin; [Thr⁴, Gly⁷]OT, oxytocin agonist; [(S)PMP₁, D-Trp₂, Pen⁶, Arg⁸]OT, oxytocin antagonist; AVP, [Arg⁸]vasopressin; HMX, hexamethonium, nicotinic receptors antagonist; gallamine triethiodide, striated muscle relaxant; PNX, bilateral section of the pelvic nerve; iv, intravenously. *Before cumulative doses of OT intrathecally (it) delivered at the L4–L6 level.
Kruskal-Wallis one-way ANOVA on ranks demonstrated that there was a treatment effect on the number of ICP rises ($H = 41.5$, df = 9, $P = 4.1 \times 10^{-6}$). Rats that received an injection of NaCl at the L4-L6 level, OT iv, and OT it at the T12-T13 level significantly displayed fewer ICP rises than rats treated with OT it at the L4-L6 level, the OT agonist it at the L4-L6 level, or OT it at the L4-L6 level after curarization ($P < 0.05$ for each). In contrast, there was no significant difference among the last three groups.

We also searched for a dose effect of OT or its agonist on the total number of ICP rises (Fig. 2). The response curve of the it OT L4-L6 group was bell shaped (Fig. 2). In this group, there was a statistically significant effect of the dose of OT injected on the number of ICP rises (Friedman repeated-measures ANOVA on ranks, $\chi^2 = 38.8$, df = 7, $P = 2.0 \times 10^{-6}$). Ten and thirty nanograms of OT elicited significantly more ICP rises than the other doses ($P < 0.05$ for each). In the group treated with the OT agonist delivered it at the L4-L6 level, the dose effect was also present (Friedman repeated-measures ANOVA on ranks, $\chi^2 = 27.8$, df = 7, $P = 2.0 \times 10^{-4}$), but the greatest doses used elicited the greatest number of ICP rises. One-hundred nanograms of the OT agonist elicited significantly more ICP rises than vehicle 0.3, 1, 3, and 10 ng ($P < 0.05$ for each). Three-hundred nanograms of the OT agonist elicited signifi-
Table 2. Effects of it and iv injections of OT and other drugs 1) on the number of rats that displayed at least one ICP rise during the experiment (responders) relative to N and 2) on the number of ICP rises per rat in each group

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route/Site</th>
<th>Responders/N</th>
<th>Number of ICP Rises/Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (0.9%)</td>
<td>it L4–L6</td>
<td>8/10</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>OT</td>
<td>it L4–L6</td>
<td>10/10</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>OT</td>
<td>it T12–T13</td>
<td>6/10</td>
<td>2 ± 2*</td>
</tr>
<tr>
<td>OT</td>
<td>iv</td>
<td>6/8</td>
<td>3 ± 3*</td>
</tr>
<tr>
<td>[Thr4, Gly7]OT</td>
<td>it L4–L6</td>
<td>8/8</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>[(S)PMP1, n-Trp2, Pen6, Arg8]OT</td>
<td>it L4–L6</td>
<td>1/8*</td>
<td>1 ± 2*</td>
</tr>
<tr>
<td>AVP</td>
<td>it L4–L6</td>
<td>1/8*</td>
<td>1 ± 1*</td>
</tr>
<tr>
<td>Pxn</td>
<td>it L4–L6</td>
<td>1/8*</td>
<td>1 ± 1*</td>
</tr>
<tr>
<td>HMX</td>
<td>iv</td>
<td>0/5*</td>
<td>0*</td>
</tr>
<tr>
<td>Gallamine triethiodide</td>
<td>iv</td>
<td>3/5</td>
<td>9 ± 10</td>
</tr>
</tbody>
</table>

N, no. of rats in each group; ICP, intracavernous pressure. *Statistically different from OT delivered it at the L4–L6 level (P < 0.05). No. of ICP rises/rat are means ± SE.

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significantly more ICP rises than vehicle 0.3, 1, and 3 ng (P < 0.05 for each).

Comparing the effects of OT and its agonist, 10 ng of OT elicited a greater number of ICP rises than 10 ng of the agonist (P = 0.0321). No difference in the number of ICP rises occurred at 30 and 100 ng between OT and its agonist (P = 0.0548 and P = 0.1584, respectively). Finally, 300 ng of the OT agonist elicited more ICP rises than 300 ng of OT (P = 0.0084).

We further measured the effects of OT and its agonist on the amplitude of the ICP rises, expressed as the ICP/BP ratio (Fig. 3). When ICP reached 40–60 mmHg, we could observe a vascular engorgement of the penis. One-way ANOVA revealed that there was a dose effect of OT on ICP/BP [F(9,79) = 7.91, P = 7.0 × 10−7]. OT (10, 30, and 100 ng) elicited ICP/BP greater than vehicle (0.3, 1, and 300 ng; P < 0.05 for each). No difference was found among 10, 30, and 100 ng OT. There was also a dose effect of the OT agonist on ICP/BP: χ² for this group was 35.5 (df = 7, P = 9 × 10−6). One-hundred and three-hundred nanograms of the OT agonist elicited ICP/BP significantly greater than those elicited by the other doses (P < 0.05 for each), and 300 ng elicited greater responses than 100 ng (P < 0.05).

Furthermore, there was a dose effect of OT injections on the AUC of the ICP rise, expressed by the AUC/BP ratio (Fig. 4; χ² = 34.0, df = 7, P = 1.6 × 10−5). OT (10 and 30 ng) elicited significantly greater AUC/BP than the other doses (P < 0.05 for each). In contrast, there was no difference between 10 and 30 ng OT. The OT agonist also yielded a significant dose effect on AUC/BP [F(7,63) = 7.81, P = 2.5 × 10−6]. One-hundred and three-hundred nanograms of the OT agonist yielded a significantly greater AUC/BP than the other doses (P < 0.05 for each), although there was no dif-
Table 3. Effect of a single it injection of OT at the L4–L6 spinal cord on 1) the number of rats that displayed at least one ICP rise during the experiment (responders) relative to N, 2) the number of ICP rises per rat in each group, 3) the latency of the first ICP rise (s), 4) the duration of ICP rises (s), 5) the amplitude of ICP at least one ICP rise during the experiment (responders) relative to N, 2) the number of ICP rises per rat in each group, 3) the latency of the first ICP rise (s), 4) the duration of ICP rises (s), 5) the amplitude of ICP rises (ICP/BP ratio), and 6) the AUC of ICP rises (arbitrary units).

<table>
<thead>
<tr>
<th>Doses, ng</th>
<th>Responders/N</th>
<th>Number of ICP</th>
<th>Latency</th>
<th>Duration</th>
<th>ICP/BP</th>
<th>AUC/BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9/12</td>
<td>4.4 ± 1.3</td>
<td>597 ± 149</td>
<td>75 ± 8.0</td>
<td>0.70 ± 0.050</td>
<td>26.9 ± 3.3</td>
</tr>
<tr>
<td>30</td>
<td>6/7</td>
<td>4.9 ± 1.4</td>
<td>696 ± 202</td>
<td>65 ± 7.0</td>
<td>0.68 ± 0.010</td>
<td>22.6 ± 3.4</td>
</tr>
<tr>
<td>100</td>
<td>6/7</td>
<td>4.7 ± 1.0</td>
<td>422 ± 129</td>
<td>90 ± 9.0</td>
<td>0.75 ± 0.1</td>
<td>35.6 ± 6.9</td>
</tr>
<tr>
<td>300</td>
<td>2/8*</td>
<td>1.0 ± 0.7</td>
<td>507 ± 216</td>
<td>52 ± 2.0</td>
<td>0.44 ± 0.03</td>
<td>9.1 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, blood pressure; AUC, area under the curve. *Statistically different from the other doses.
receptor present in uterine smooth muscle (2); a difference between spinal and peripheral OT receptors could explain why 300 ng OT does not elicit any erectile response in our experiment. It is unlikely that the saturability of the OT receptor accounts for the lack of ICP rises on 300 ng OT, because this dose should elicit at least as many ICP rises as 100 ng OT. It is interesting to note that 300 ng OT elicited no more ICP rises when injected as either cumulative or single doses. Could the desensitization of the OT receptor explain this decrease of ICP response? In cultured astrocytes, OT applications elicited calcium rises in which amplitude decreased if the application was repeated. The authors observed a 20-min wash period between two applications before they could record a full recovery of the calcium response (10). In our experiment, a period of 15 min separated two consecutive injections, which suggests that no recovery could occur in this condition. OT (10, 30, and 100 ng) elicited fewer ICPs on single doses compared with cumulative doses. Furthermore, the ICP/BP ratio displayed a bell-shaped response curve for cumulative treatments, but no such profile was noted on single-dose treatments. It remains unclear to us whether such differences rely on time of exposure of the receptor to OT or to an interaction between time of exposure and dose.

At high doses, OT may bind vasopressin receptors. The latter are present in the lumbosacral spinal cord of rats (33). Although 10 times less potent than vasopressin, OT could act on the V1 receptor of sympathetic preganglionic neurones of the neonate rat spinal cord (27). In our experiment, OT acting at vasopressin receptors could display inhibitory effects on spinal proerectile neurones. We tested the effects of it vasopressin. The peptide had no effect on ICP. In contrast, it could oppose a proerectile effect of OT.

SPN neurons convey parasympathetic fibers to the penis through the pelvic and cavernous nerves (14, 18, 21). Electrical stimulation of the pelvic and cavernous nerves elicits ICP rises in anesthetized rats (12, 13, 28). By delivering HXM iv, we blocked nicotinic receptors, thereby inhibiting the synaptic transmission between preganglionic fibers of the pelvic nerve and postganglionic fibers in the cavernous nerve. After HXM injection, no ICP rise occurred in response to it OT, demonstrating that OT recruited preganglionic neurones. The bilateral section of the pelvic nerve also prevented any ICP increase to occur after OT injection. Therefore, in the present study, the proerectile effects of OT are caused by activation of the sacral parasympathetic outflow. However, oxytocinergic activation of sacral parasympathetic pathways may be direct or relayed through interneurons present in the dorsal grey commissure of the lumbosacral spinal cord. Tight anatomic relationships between the two areas in the rat spinal cord, as demonstrated with transsynaptic transport of PRV from the corpus cavernosum, confirm this hypothesis (18). Furthermore, both areas contain OT receptors (34). Therefore, OT released by descending PVN-spinal pathways may activate both interneurons and preganglionic neurones.

Penile erection in conscious mammals recruits autonomic pathways to the penis and somatic pathways to the perineal striated muscles (25). In conscious mammals, contraction of striated muscles on the erect penis elicits peaks of penile pressure rises that largely override BP (5, 23, 26). According to some authors, bulbospongious (BS) and ischiocavernous (IC) motoneurons receive descending projections from the PVN (35). Furthermore, transsynaptic retrograde labeling from the IC or BS muscles using PRV or rabies virus labels some neurones in the PVN (19, 31). According to these data, OT could also control the somatic outflow to the perineal striated muscles. However, although rare OT-immunoreactive fibers have been demonstrated in the ventral horn of the rat spinal cord (30, 34), this area does not contain OT receptors (34). After OT injection, we never recorded ICP rises over BP, and the injection of the striated muscle blocking agent gallamine triethiodide did not affect the ICP rises elicited by OT. Therefore, our data demonstrate an effect of OT on penile pressure, independent of striated muscle, and suggest a lack of excitatory effect of OT onto IC and BS motoneurons.

OT may activate lumbosacral parasympathetic neurones and interneurons destined to pelvic organs other than the penis. Indeed, it was demonstrated that in conscious female rats, it OT increased micturition pressure and decreased bladder capacity and micturition volume (22). Interestingly, the most efficient dose in this model was 30 ng OT. We also identified 30 ng as the dose of OT that yielded the greatest probability of eliciting ICP rises when injected in cumulative doses, and only the number of responders when OT was injected as single doses. If comparable doses of OT activate different parasympathetic outflows, then it remains to be determined how the spinal network integrates this increase of OT, because all pelvic viscera are not active at the same time. It may be suggested that in a sexually relevant context, it is the convergence of information from the periphery and from supraspinal structures that elicits the specific activation of proerectile pathways at the spinal cord level.

In conscious rats, noncontact erections and drug-induced erections (e.g., apomorphine-induced erections) reflect the activity of supraspinal nuclei. These erections are transient and repetitive, and recording ICP during noncontact and apomorphine-induced erections revealed transient rises of ICP (6). In our experiments, it injections of OT elicited phasic ICP rises. It suggests that the spinal cord translates the tonic excitation by supraspinal nuclei or by OT into the phasic activation of parasympathetic pathways leading to phasic ICP rises.

In our experiment, OT would be a potent activator of the spinal generator of penile erection. Once this rhythmic generator is activated, OT could not further regulate the number, the amplitude, and the area of the ICP rises, as evidenced by lack of dose effect of OT injection on the ICP/BP and AUC/BP ratios.
Pharmacological stimulation of the PVN in conscious rats and its electrical stimulation in anesthetized rats elicit penile erection (4, 8). Lesions of the PVN suppress apomorphine-induced erections (4) and impair noncontact erections (16). In rats, fibers issued from the parvocellular part of the PVN reach the lumbosacral spinal cord (7). The SPN contains OT-immunoreactive fibers and OT receptors (30, 34). Our results suggest that by delivering OT at the lumbosacral level, we mimicked the release of OT by PVN-spinal fibers in rats. The present results demonstrate that OT exerts proerectile effects, as measured through increases in ICP, when it is delivered at the L4-L6 spinal cord in anesthetized rats. They demonstrate that the effects are specific, being mimicked by a specific agonist but not by arginine-vasopressin, and are blocked by a specific OT antagonist. Proerectile effects of OT are due to the activation of autonomic efferent pathways running in the pelvic nerves.

Perspectives

Our experiments suggest that the lumbosacral spinal cord is the final target of a proerectile, oxytocinergic pathway in which perikarya are in the parvocellular part of the PVN. This pathway represents a very efficient and direct proerectile link between supraspinal nuclei and the spinal cord. To better understand the contribution of peripheral and supraspinal information to the generation of erection, it is tempting to test the effects of OT in rats after a complete section of the spinal cord at the thoracic level, i.e., the interruption of the responses of the spinal cord to OT after a specific OT antagonist. Proerectile effects of OT are measured through increases in ICP, when it is delivered at the L4-L6 spinal cord in anesthetized rats. They demonstrate that the effects are specific, being mimicked by a specific agonist but not by arginine-vasopressin, and are blocked by a specific OT antagonist. Proerectile effects of OT are due to the activation of autonomic efferent pathways running in the pelvic nerves.

REFERENCES


