The effects of intrathecal administration of the dopamine agonist apomorphine on penile reflexes and copulation in the male rat

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Abstract. Relatively high doses of systemically administered apomorphine inhibit penile reflexes. It is possible that these inhibitory effects are due, at least in part, to actions of apomorphine on the lumbosacral spinal cord. The present experiments examined this possibility by injecting apomorphine (10 and 50 µg/5.0 µl vehicle) into the lumbosacral subarachnoid space through chronic, indwelling cannulae. Such injections impaired ex copula penile reflexes, slowed the rate of copulation, and decreased the number of intromissions preceding ejaculation. These results suggest that lumbosacral cord dopamine receptors may normally regulate male sexual performance.

Key words: Apomorphine – Dopamine – Penile reflex – Sexual behavior – Spinal cord – Rat

Systemic administration of the dopamine (DA) agonist apomorphine (APO) has been shown to produce a biphasic effect on penile reflexes when tested ex copula in the rat (Benassi-Benelli et al. 1979; Serra et al. 1983; Gower et al. 1984; Pehek et al. 1988). Low to moderate doses facilitated, whereas higher doses inhibited, penile reflexes. Administration of moderate doses of APO also facilitated ejaculation in copula, as evidenced by a decrease in the ejaculatory threshold (i.e., a decrease in the number of intromissions preceding ejaculation and a shortening of the latency to ejaculate) (Tagliamonte et al. 1974; Paglietti et al. 1978; Ahlenius and Larsson 1984; Napoli-Farris et al. 1984) whereas higher doses impaired copulation (Clark and Smith 1987). These effects were due to actions on central, as opposed to peripheral, DA receptors since the effects on penile reflexes and the facilitative effects on copulation were blocked by pretreatment with centrally acting DA antagonists (e.g., haloperidol) but not by the non-centrally acting antagonist domperidone (e.g., Falaschi et al. 1981; Ahlenius and Larsson 1984; Pehek et al. 1988).

It is possible that APO’s biphasic effect on penile reflexes results from differential binding to anatomically separate populations of DA receptors by lower and higher doses. For example, such a situation could result from differences in the pharmacokinetic distribution of APO to different central nervous system (CNS) sites. We have recently shown that the facilitation of penile reflexes produced by systemic administration of APO may be at least partially due to actions on the medial preoptic area. The inhibition of penile reflexes produced by systemically administered APO may result from actions on DA receptors in the lumbosacral spinal cord. This area of the cord receives sensory input from the penis and contains the parasympathetic and somatic motor nuclei that project to the penile vasculature and perineal muscles (the sacral parasympathetic nucleus and the spinal nucleus of the bulbocavernosus, respectively) (Arnold and Gorski 1984; Nadelhaft and Booth 1984). DA neurons that originate in cell group A11 of the caudal hypothalamus and travel within the diencephalospinal DA pathway innervate the lumbosacral cord (Skagerberg et al. 1982; Skagerberg and Lindvall 1985).

The present studies examined the effects of intrathecal administration of the DA agonist APO on penile reflexes and copulation. It was hypothesized that penile reflexes would be inhibited following APO injections into the lumbosacral subarachnoid space.

Methods

Animals. Adult male Long-Evans rats weighing from 350 to 450 g were used. Animals were housed singly with food and water available ad lib. Ovariectomized female rats, housed separately from the males, were brought into behavioral heat with a single SC injection of estradiol benzoate (20 µg) administered 48 h before behavioral testing.

Surgery and cannulae. The intrathecal implantation procedure utilized was adapted by Sachs (University of Connecticut) from the procedure described by LoPachin and Rudy (1981). Briefly, a small hole was punctured through the atlanto-occipital membrane and a cannula constructed of polyethylene tubing was then inserted into the puncture and lowered into the spinal subarachnoid space, terminating in the desired spinal cord segment. The exposed end of the cannula was threaded between two screws that were anchored in the skull, and dental cement surrounded this assembly.

Cannulae were constructed from PE-10 polyethylene tubing. The diameter of the section of the cannula to be inserted was reduced by stretching it to 140% of its original length in a bath of hot water. The stretched portion was cut to 8.5 cm for a lumbosacral cannula and 4.5 cm for

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a midthoracic cannula. An obturator fashioned from 30 ga
stainless steel tubing prevented the entry of foreign material
into the cannula.

For those animals in penile reflex experiments, each
male’s suspensory ligament was excised at the time of can-
num implantation. This ligament is attached bilaterally to
the base of the penis and serves to cause retraction of the
penis back into the penile sheath after protrusion. Severing
the ligament facilitated maintained exposure of the penis
from the sheath during penile reflex tests.

Drug administration. Apomorphine HCl (Sigma, St. Louis,
MO) was dissolved in sterile water with 0.2% ascorbate.
A Harvard microinfusion pump equipped with a 1 ml syr-
inge was used to inject at a rate of 1 μl/min. A 5 μl aliquot
of drug or vehicle was injected, followed by a saline flush
(7 μl for a lumbosacral cannula and 5 μl for a midthoracic
implant). Injection rate was 1 μl/min. Behavioral testing be-
egan 15 min following the termination of the injection.

Penile reflex tests. Each rat was restrained in a supine posi-
tion in a metal restraining device (circumference, 8.5 by
5.5 cm; length, 20 cm). In order to evoke penile reflexes,
the penile sheath was retracted and maintained in this posi-
tion. Usually, reflexes spontaneously occurred within
5–10 min following exposure of the glans. The resultant
responses occurred in discrete clusters, separated by 15 s
or longer. Within a cluster, usually two to six reflexes were
displayed, with a duration from 0.5 to 2 s for each reflex.
Two major classes of responses, erections and flips, oc-
curred. Three gradations of glans erections were scored:
E1, engorgement of just the base of the glans; E2, tumes-
cence involving both the base and the tip of the glans;
E3 (also termed a cup), engorgement involving the base
as well as an intense flaring of the tip of the glans so that
the diameter of the tip was greater than that of the base
of the glans. Flips (anteroflexions of the penis) were clas-
sified as “partial” or “full”. A flip was classified as a “full
flip” if the penis traveled past the line perpendicular to
the rat’s body. These responses were recorded with the aid
of an Esterline-Angus event recorder. A test lasted 15 min
from the first reflex or 20 min if no reflexes occurred.

Copulatory behavior tests. Each copulatory behavior test
lasted 30 min from the first intromission or for a total of
30 min if no intromissions occurred. Measures derived from
the data were: the numbers of ejaculations, mounts, and
intromissions, the latencies to the first mount and intro-
mission of a test, the ejaculation latency (time from the
first intromission of an ejaculatory series to the subsequent
ejaculation), the postejaculatory refractory period (time
from the ejaculation to the following intromission), the in-
terintromission interval (the average time between inter-
missions), and the intromission ratio (the number of in-
tromissions divided by the total number of mounts plus
intromissions).

Cannulae verification. Following the completion of each ex-
periment, the rats were sacrificed and their spinal cords
were visually examined for proper cannulae placements.
Only those animals with correct placements were included
in data analyses.

Statistical analyses. All experiments employed counterba-
lanced, repeated measures designs. The data were analyzed

![Lumbosacral vs. Midthoracic Reflexes](image1)

**Fig. 1.** Effects of apomorphine injections into the lumbosacral or
midthoracic subarachnoid space on the percentage of animals
displaying penile reflexes. * P<0.01 relative to 0 (vehicle)

![Lumbosacral vs. Midthoracic Reflexes](image2)

**Fig. 2.** Effects of apomorphine injections into the lumbosacral or
midthoracic subarachnoid space on the total number of reflexes
(means ± SEM). * P<0.01 relative to 0 (vehicle). + P<0.05 rela-
tive to 50 μg. ++ P<0.01 relative to 50 μg

by repeated measures ANOVAs, followed by Newman-
Keuls post hoc comparisons. Ordinal data were analyzed by
Cochran Q tests, followed by McNemar post hoc com-
parisons.

Procedures. Testing was done during the dark period, be-
tween 11.00 and 17.00 hours. In all experiments, each rat
received vehicle, 10 μg APO, and 50 μg APO on separate
tests. Tests were given at 1-week intervals. The first experi-
ment examined the effects on penile reflexes of APO admin-
istered into the lumbosacral subarachnoid space (n=13).
In order to examine the site specificity of APO’s effects
on reflexes, rats received APO injections into the subara-
chnoid space of the midthoracic cord in the second experi-
ment (n=15). The third experiment examined the effects on copu-
latory behavior of lumbosacral APO administration (n=19).

Results

First experiment

Administration of 50 μg APO into the lumbosacral sub-
arachnoid space decreased the percentage of rats displaying
penile reflexes [Q(2)=6.25, P<0.05; see Fig. 1]. This dose
Table 1. Effects of lumbosacral intrathecal apomorphine injections on penile reflexes

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>10 µg</th>
<th>50 µg</th>
<th>794.15 ± 132.71</th>
</tr>
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<tbody>
<tr>
<td>RL</td>
<td>436.62 ± 118.11</td>
<td>535.38 ± 117.70</td>
<td>3.00 ± 1.23</td>
<td></td>
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<tr>
<td>CT</td>
<td>8.85 ± 1.53</td>
<td>6.77 ± 1.45</td>
<td>9.00 ± 3.67</td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>24.23 ± 4.17</td>
<td>16.54 ± 3.41</td>
<td>3.62 ± 1.28</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>10.31 ± 1.75</td>
<td>6.38 ± 1.26</td>
<td>8.60 ± 2.57</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>13.77 ± 2.63</td>
<td>10.08 ± 2.32</td>
<td>8.60 ± 1.28</td>
<td></td>
</tr>
<tr>
<td>FT</td>
<td>9.15 ± 2.23</td>
<td>7.15 ± 2.55</td>
<td>6.40 ± 1.22</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>6.85 ± 1.80</td>
<td>5.92 ± 2.10</td>
<td>4.60 ± 1.42</td>
<td></td>
</tr>
</tbody>
</table>

Values are the means ± SEM. RL latency to the first reflex (s); CT total number of clusters; ET total number of erections; E1 number of E1s; E2 number of E2s; FT total number of flips; PF number of partial flips

* P<0.05 relative to vehicle
** P<0.01 relative to vehicle
+ P<0.05 relative to 50 µg

Table 2. Percent change from control levels following intrathecal apomorphine injections into either the lumbosacral or midthoracic cord

<table>
<thead>
<tr>
<th></th>
<th>Lumbosacral</th>
<th>Midthoracic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µg</td>
<td>50 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>-29%</td>
<td>-65%</td>
</tr>
<tr>
<td>CT</td>
<td>-24%</td>
<td>-66%</td>
</tr>
<tr>
<td>ET</td>
<td>-32%</td>
<td>-63%</td>
</tr>
<tr>
<td>E1</td>
<td>-38%</td>
<td>-65%</td>
</tr>
<tr>
<td>E2</td>
<td>-27%</td>
<td>-61%</td>
</tr>
<tr>
<td>FT</td>
<td>-22%</td>
<td>-69%</td>
</tr>
<tr>
<td>PF</td>
<td>-14%</td>
<td>-64%</td>
</tr>
</tbody>
</table>

– and + signs refer to a decrease and an increase, respectively, in the measures

Values are derived from the means. RT total number of reflexes; CT total number of clusters; ET total number of erections; E1 number of E1s; E2 number of E2s; FT total number of flips; PF number of partial flips

also decreased the total number of reflexes \(F(2,28)=7.39, P<0.005\); see Fig. 2] and clusters \(F(2,24)=8.28, P<0.005\); see Table 1]. The decrease in reflex total was due to a decrease in both the number of erections \(F(2,24)=8.08, P<0.005\) and \(F(2,24)=4.82, P<0.005\) (see Table 1). Among erections, the number of E1s was decreased by either 10 or 50 µg APO \(F(2,24)=11.05, P<0.001\), while the number of E2s was reduced only following 50 µg APO \(F(2,24)=4.70, P<0.025\) (see Table 1). Among flips, the number of partial flips was reduced by 50 µg APO \(F(2,24)=3.55, P<0.05\); see Table 1]. Seminal emission was not affected.

Second experiment

Midthoracic APO injections did not affect the percentages of animals displaying reflexes (see Fig. 1). The effects of 50 µg APO administered into the midthoracic subarachnoid space were diminished relative to the effects produced by the same dose in the lumbosacral cord (see Table 2). Furthermore, while there was a trend towards an inhibition of penile reflexes following 10 µg lumbosacral APO (statistically significant in the case of E1s), no such trend was evident following 10 µg midthoracic APO (see Table 2).

Administration of 50 µg midthoracic APO decreased the total number of reflexes \(F(2,28)=9.41, P<0.001\); see Fig. 2], clusters \(F(2,28)=7.01, P<0.005\), and erections \(F(2,28)=9.69, P<0.001\) (see Table 3). This dose did not significantly affect the total number of reflexes, although the number of partial reflexes was decreased \(F(2,28)=7.30, P<0.005\) (see Table 3). Among erections, 50 µg APO decreased the number of E1s \(F(2,28)=10.61, P<0.001\] while there was a trend towards an increased number of E1s following 10 µg APO \(F(2,28)=3.18, 0.05<P<0.1\) (see Table 3). Seminal emission was not affected.

In order to statistically compare the data from this experiment with that from experiment 1, difference scores were obtained from the raw data by subtracting vehicle data from drug data. Analyses of these scores revealed that, following 50 µg APO, fewer lumbosacral rats displayed reflexes \(\chi^2(1)=6.92, P<0.01\) and these rats had fewer E1s \(t(26)=2.19, P<0.05\) than midthoracic animals. After 10 µg APO, lumbosacral rats had fewer total reflexes \(t(26)=2.26, P<0.05\), fewer total erections \(t(26)=2.54, P<0.02\] and fewer E1s \(t(26)=3.92, P<0.001\] than midthoracic rats.

Third experiment

Seven males failed to ejaculate on one trial of the study (two following vehicle administration, two following 10 µg APO, and three following 50 µg APO). Excluding these animals from data analyses due to missing data points would have reduced the sample size to 12. Thus, for each measure within a given treatment, the arithmetic means of those animals that did ejaculate under that treatment were substituted for the missing data points. The data were then analyzed both with and without these substitutions. The same trends were apparent following both types of analyses but were only statistically significant when data from all 19 animals were utilized. The following results are based on analyses of all 19 animals.

Intrathecal administration of either the 10 or 50 µg dose of APO reduced the number of intermissions in the first ejaculatory series \(F(2,36)=5.23, P<0.025\], lengthened the interejaculatory interval of this series \(F(2,36)=3.51, P<
Table 4. Effects of intrathecal apomorphine injections on male copulatory behavior

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>10 µg</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>10.82 ± 0.75</td>
<td>8.82 ± 0.51</td>
<td>8.12 ± 0.72</td>
</tr>
<tr>
<td>III1</td>
<td>40.99 ± 3.38</td>
<td>55.86 ± 7.27</td>
<td>60.22 ± 10.73</td>
</tr>
<tr>
<td>PEI1</td>
<td>439.76 ± 16.08</td>
<td>478.06 ± 19.60</td>
<td>514.87 ± 27.95</td>
</tr>
<tr>
<td>EL2</td>
<td>190.87 ± 13.67</td>
<td>270.20 ± 29.73</td>
<td>246.25 ± 16.99</td>
</tr>
</tbody>
</table>

Values are the means ± SEM for significantly affected variables. N1, number of intromissions preceding the first ejaculation; III1, first intromission interval; PEI1, first postejaculatory interval; EL2, second ejaculation latency

* P < 0.05 relative to vehicle

0.05], and increased the latency to the second ejaculation [F(2, 36) = 3.52, P < 0.05] (see Table 4). The postejaculatory interval following the first ejaculation was lengthened by 50 µg APO [F(2, 36) = 4.27, P < 0.025; see Table 4]. There were no differences between treatments in the percentages of animals mounting, intromitting, or ejaculating. Motoric behavior following APO administration appeared normal.

Discussion

Administration of APO into the lumbosacral subarachnoid space produced a dose-dependent inhibition of penile reflex potential, slowed the rate of copulation (as seen by a lengthening of the interintromission interval, the ejaculation latency, and the postejaculatory interval), and decreased the number of intromissions preceding ejaculation. These effects appear to be due to actions on the lumbosacral cord, rather than on other cord levels or the brain, since the inhibition of penile reflexes was abolished or diminished when APO was administered into the midthoracic subarachnoid space. Furthermore, the observed effects were opposite to those seen following the intracranial administration of APO in previous studies (Hull et al. 1986; Pehek et al. 1989).

The present results do not appear to be secondary to non-specific alterations in general motor activity. Intrathecal APO injections did not produce any noticeable alterations in general activity and did not interfere with either the latency to begin copulation or the number of animals copulating, suggesting that the subjects were motorically normal. Thus, the present findings appear to be due to a relatively specific effect of APO on male reproductive behavior and suggest that DA receptors in the lumbosacral cord normally inhibit male sexual performance, including reflexive penile responses.

Sachs (1983) has provided evidence indicating that the penile reflexes observed in the restrained, supine rat are utilized during copulation and that different peripheral mechanisms may subserve the different reflexes. Flips and cups were associated with intromission and ejaculation (respectively) and appeared to result primarily from activity in the striated perineal musculature. Erections were primarily a function of autonomic input to the penile vasculature. In the present experiments, both erections and flips were inhibited by intrathecal APO administration, suggesting that DA receptors in the lumbosacral cord regulate both the vascular and somatic mechanisms involved in the production of penile reflexes. Seminal emission was not affected by intrathecal APO administration, supporting previous findings suggesting that experimentally separable populations of DA receptors may regulate penile reflexes and seminal emission (Pehek et al. 1988).

As previously mentioned, we have shown that the facilitation of penile reflexes produced by IP administration of lower doses of APO (0.1 mg/kg) may be mediated (at least in part) by actions on the medial preoptic nucleus (Pehek et al. 1989). Injections of 1.0 or 2.0 µg APO/0.5 µl (a 2:1 or 4:1 drug to vehicle ratio, respectively) into the preoptic area enhanced ex copula penile reflex potential (Pehek et al. 1989) and facilitated copulatory behavior in tests with a female (Hull et al. 1986). In more recent work, 10.0 µg APO/0.5 µl vehicle (a 20:1 ratio) in the preoptic area also facilitated copulation (Hull et al. 1989). In contrast, the present results demonstrate that intrathecal injections of APO at concentrations (10 or 50 µg APO/5 µl vehicle, a 2:1 or 10:1 ratio, respectively) comparable to those used in our preoptic area studies inhibited penile reflexes and copulation. Higher doses (0.5 mg/kg) of systemically administered APO also inhibit penile reflexes (Pehek et al. 1988). Our work suggests that this inhibition is not due to actions on the medial preoptic nucleus. Rather, the impairment following systemic APO may be at least partially due to actions on the lumbosacral cord. However, it is difficult to compare doses of APO administered directly into brain tissue with those administered intrathecally or systemically since the relevant concentrations at the DA receptor are unknown. Further dose-response studies would help to clarify this issue.

Copulation in the male rat has been conceptualized as consisting of both motivational and performance components (Beach 1956; Sachs 1978). Intrathecal APO administration affected measures of copulatory performance (e.g., penile reflexes, the interintromission interval) but did not affect measures of sexual arousal (e.g., mount and intromission latencies). These results suggest that DA receptors in the lumbosacral cord regulate copulatory performance, rather than sexual motivation, and support the hypothesis, made by others (Beach 1956; Sachs 1978), that the components comprising male sexual behavior are regulated by relatively independent neural mechanisms.

Previous studies examining the role of spinal cord DA in the regulation of male sexual behavior have employed drugs that are not specific to the DA system (Hansen 1982; Stefanick et al. 1982; Svensson and Hansen 1984). Systemic administration of the DA and 5-HT receptor agonist RDS-127 abolished the facilitation of penile reflexes seen after spinal transection and induced seminal emission in the same rats, suggesting a spinal site of action for these effects (Stefanick et al. 1982). The inhibition of penile reflexes seen in the present experiments agrees with the above report and suggests that the dopaminergic properties of RDS-127 may have been responsible for its effects on reflexes. However, unlike the study by Stefanick et al., seminal emission was not affected in the present experiments. It is possible that the RDS-induced facilitation of seminal emission was due either to actions on peripheral mechanisms or to actions on 5-HT receptors.

The present results suggest that endogenously released lumbosacral cord DA inhibits the display of penile reflexes. This DA may be released from the terminals of diencephalosplinal dopamine neurons and may be at least partially
responsible for the tonic supraspinal inhibition of penile reflexes that has been observed (Hart 1968). These neurons also appear to regulate the rate of copulation and perhaps the number of intromissions preceding ejaculation as well. DA receptors in the lumbosacral cord may integrate reflexive penile responses with other aspects of male copulatory performance.

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