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Cholinergic facilitation of erection and ejaculation in spinal cord-transected rats

VM Vargas[1], D Torres[1], F Corona[1], M Vergara[1], LE Gómez[2], R Delgado-Lezama[3] and R Cueva-Rolón[2]*

[1]*Departamento de Agrobiología, Universidad Autónoma de Tlaxcala, Tlaxcala, Mexico;* [2]*Laboratorio de Tlaxcala, Centro de Investigacion y Estudios Avanzados, Tlaxcala, Mexico; and* [3]*Departamento de Fisiologia, CINVESTAV del I.P.N., Tlaxcala, Mexico*

Penile reflexes (PRs) were monitored in chronic spinal cord-transected rats by identifying them visually, and at the same time they were recorded as the electromyographic activity of bulbospongiosus muscles. Intraperitoneal injection of the agonist muscarine (10 μg) produced a facilitation of PRs. A decrease in the latency, an increase in the number of clusters and often an increase in the duration of cups were found after muscarine. In addition, 66% (six out of nine) of the animals ejaculated after muscarine. These results suggest that cholinergic receptor stimulation may be involved in erectile and ejaculatory mechanisms mediated by the spinal cord.
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Introduction

The stimulation of spinal cord acetylcholine receptors has been reported to facilitate ejaculation. For example, ejaculation is stimulated by cholinesterase inhibitors in spinal cord-injured men:[1,2] intrathecally applied neostigmine stimulates penis erection and ejaculation in 58% of subjects,[1] and ejaculation is found in 40% of subjects when physostigmine is given systemically.[2] In addition, in freely moving rats, the stimulation of the spinal M-type cholinergic receptors facilitates sexual behavior, since a decrease of ejaculatory latency, intromission frequency and intromission interval is found when the agonist muscarine is applied intrathecally. On the contrary, when the muscarinic receptor antagonist homatropine is applied, a decrease in the number of animals that are able to copulate is found in sexually trained and vigorous males.[3] However, it is unclear how the activation of the spinal cord muscarinic receptors facilitates ejaculation; the stimulation of lumbar reflex centers has been suggested.[3,4]

In addition, it has been found that in spinal cord-transected rats muscarine produces glans penis erections that often accompany rhythmic discharges in the bulbospongiosus (BS) muscles. BS discharges elicited by muscarine had a similar electromyographic (EMG) activity to that found in the urethro genital reflex (UGR).[5] UGR is an experimental model of the sexual climax that can be evoked in response to the mechanical stimulation of the urethra, and consists of glans penis erections, rhythmic discharges in BS muscles and expulsion of the urethral contents.[6,7] These evidences strongly suggest a cholinergic participation in the ejaculatory mechanism, and they suggest a facilitation of the erectile potential. However, an adequate description of the erectile response to the stimulation of spinal muscarinic receptors is missing.

Penile reflexes (PRs) are routinely used for testing the erectile potential in unanesthetized male rats. PRs are evoked in males lying on their back after retracting the penile sheath, and are characterized by four different responses: (i) erections: reddening and distension of the penile glans; (ii) cups: trumpet shape type of erections; (iii) flips: quick anteroflexions of the glans; and (iv) long flips: large and quick flips with the glans at an angle of 90–110°. Clusters of erections, cups and flips occur at 1–2 min intervals and can continue for an hour (see, for review, Meisel and Sachs[32]).

PR can be elicited from chronic spinal cord-transected subjects both in men[8] and in experimental animals,[9–12] and they are facilitated by the spinal cord transection, since the latency to the first cluster is reduced and the number of genital responses (erections, cups and flips) is significantly larger than in control animals.[9,13] Thus, it can be tested whether the erectile potential may also be affected by

*Correspondence: R Cueva-Rolon, Centro de Investigacion y Estudios Avanzados, Laboratorio de Tlaxcala, Plaza Hidalgo s/n, Panotla, Tlaxcala 90140, Mexico.
E-mail: laboratorio@servired.com.mx
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stimulating the muscarinic cholinergic receptors in spinal cord-transected rats. This is the scope of the present investigation.

Material and methods

Experiments were carried out in nine male Wistar rats sexually naive (350–400 g) from our facilities. Three rats per cage were housed in an inverted light cycle (lights on 2200; off 1000) with *ad lib* access to food and taped water. Room temperature was kept at 28°C and humidity at 60%.

Spinal cord transection was performed as reported previously.[14,15] Briefly, under barbiturate anesthesia (pentobarbital, 35 mg/kg, intraperitoneal (i.p.)), the T6 dorsal spinal process was removed to expose the spinal cord. The dura was cut and the spinal cord was transected with a blunt spatula. The spinal cord transection was verified visually with the aid of a Zeiss surgical microscope and Gelfoam was inserted to prevent bleeding, after which the overlying muscle and skin were sutured. The animals were wrapped in an electric blanket until recovery from anesthesia and housed individually.

As in previous experiments,[14] in order to maintain the subjects in healthy condition after spinal cord transection, the rats were washed of the ventrum with running water, towel drying and the urine was expressed manually three times a day for the first 2 weeks after the surgery. In addition, oxytetracycline (17 mg/kg) and gentamicin sulfate (5 mg/kg) were given i.m. twice daily to prevent infections of the urinary tract. After the third week, the animal care was administered once daily, since animals had recovered their voiding function.

Rats were tested for the presence of PRs during the third week after surgery. PR were elicited in a manner similar to the method described earlier.[9] Rats were restrained on their back with a Velcro belt, while the anterior portion of their body was enclosed in a glass cylinder. The penile sheath was pushed behind the glans and held in position with a glass rod throughout the test. Test ended 20 min after retraction of the penile sheath, and only one test was carried out for every observation.

A base line was set for each subject by recording the responses every 2 days during the third week after transection. Once the base line was set, two groups were made at random (M, $n=5$; and S, $n=4$) for a crossover experiment design. On the fourth week, muscarine (muscarine hydrochoride, Sigma; 10 μg in 0.5 ml saline i.p.) was applied to M group and saline (0.5 ml i.p.) to S group. PR were tested 5 min after the injection of the drug or saline. After 4 days of resting, saline was applied to those animals that received muscarine, and muscarine to those receiving saline. The muscarine concentration was chosen from the dose/response curve from previous observations.[5,16]

The ventral BS covers the ventral surface of the penile bulb,[17] and it can be easily located by pushing the testicles laterally. Once the rats were restrained, a pair of platinum needles (Grass S2; Grass Instruments) was inserted into the distal portion of BS, just beneath the penile bulb, for recording their EMG activity. The electrode location was verified by recording BS reflex response to the stimulation of the glans[18] before retracting the penile sheath.

EMG signals were amplified (CP511 AC amplifier, Grass Instruments) and were connected to an analogical–digital board (Digidata 1200A; Axon Instruments). Data were stored in a PC computer for later analysis. The presence of glans erections, cups, penile flips and long flips were recorded with the time tag button of the acquisition program (Axoscope; Axon Instruments). The latency to the first penile response, usually a glans erection, was measured and the number of penile clusters and the events within the clusters for every animal were counted. Data were averaged and a comparison between M and S groups were carried out by using one-way ANOVA test, with the Newman–Keuls test for pairwise comparisons. A value of $P<0.05$ was used as statistically significant.

Results

PRs could be evoked in the spinal cord-transected subjects a week after surgery;[9,13] however, in the present observations two more weeks of recovery were allowed before setting the base line. As reported previously,[9,13] several seconds after exposing the glans penis a series of genital response clusters appeared in all nine animals. Usually, each cluster started with an erection and included three or four erections, two or three cups and one or two flips or long flips. The end of a response cluster can be easily identified because PR remained quiescent until the beginning of another response cluster 1–2 min after the onset of the preceding one.

A significant increase in the number of reflex clusters was found after muscarine, compared to saline. The changes of PR after muscarine are shown in Figure 1. As shown in the example of Figure 1a, the number of reflex responses after muscarine was significantly higher compared to those found after saline. The records of BS activity at expanded scale shown in Figure 1b are similar to those reported previously.[17] The background activity of BS showed no change in response to muscarine in seven out of nine subjects. In the two remaining subjects, an increase of background activity was produced by the drug. Similar responses were seen after the injection

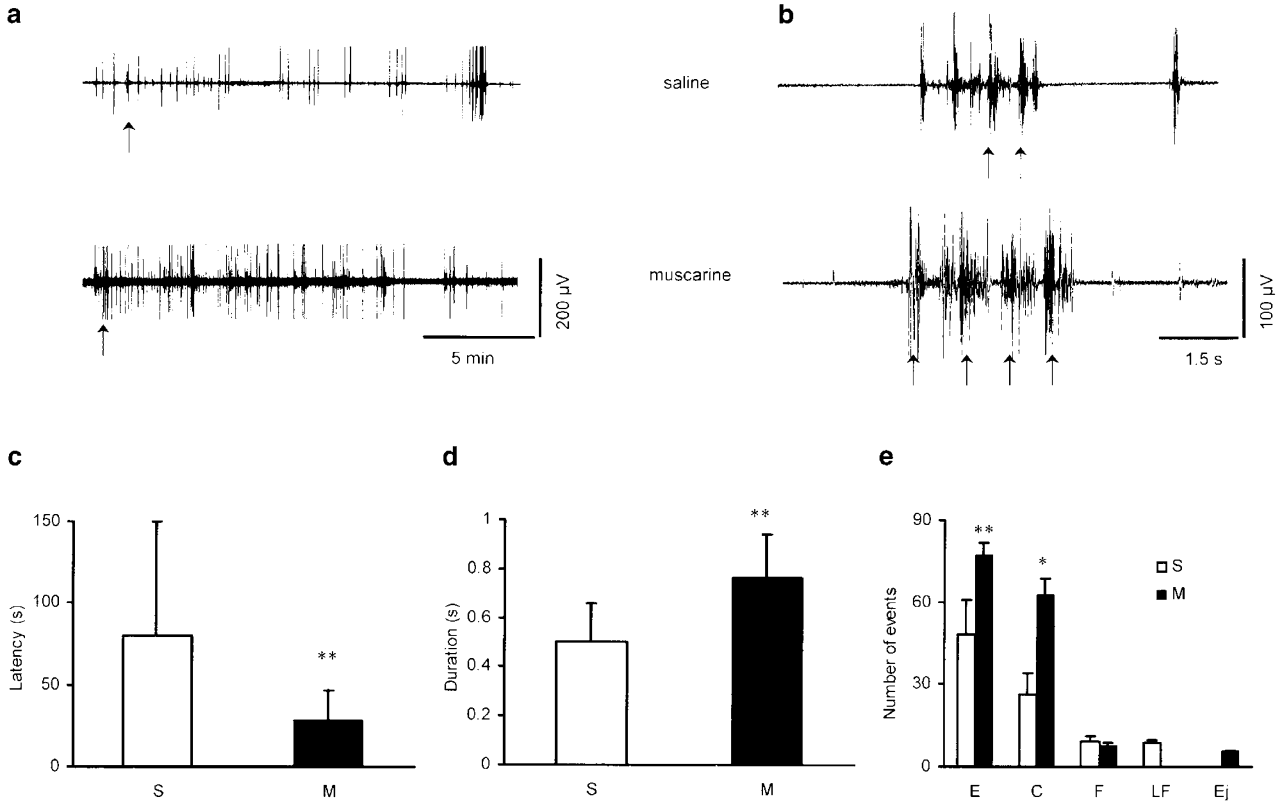

Figure 1 Changes of PRs produced by muscarinic receptors stimulation. (a) EMG recordings of BS muscle for the duration of the 20 min test. Note the increase in the number of reflex responses after muscarine. Recordings are from the same rat. A larger background activity is present after muscarine compared to that found after saline (see b). (b) EMG activity of a selected cluster (arrow in a) at expanded time base. Arrows point to penile cup episodes. As shown, not only the number but also the duration of penile cups are increased by muscarine. Records are taken from the fourth clusters of (a). In (a, b) top trace saline; bottom trace muscarine. (c) Latency to the first glans erection. (d) Duration of penile cups. (e) Number of glans erections (E), cups (C), flips (F) long flips (LF) and ejaculation (Ej). Data show the total number of events during the 20 min period. In C–E, data are expressed as the mean ± s.d. Saline, open bars; Muscarine, filled bars. $*P<0.001$, $**P<0.01$, Newman–Keuls test.

of muscarine, irrespective of whether the drug was injected before or after the saline.

Even though there was a great individual variation in the latency to the first cluster (Figure 1c), a shorter latency to the first glans erection was seen after muscarine. After muscarine, the penile clusters as well as the penile cups per cluster were more frequent (Figure 1a). The number of erections and penile cups were also significantly larger compared to saline (Figure 1e); however, the number of glans erections per cluster were not different between treatments (Table 1). Furthermore, as shown in Figure 1b, the duration of penile cups were significantly longer after muscarine compared to saline. No difference was found in the number of penile flips between treatments. No long flip was present in any of the animals after muscarine (Figure 1e). The data from the M and S groups are summarized in Table 1.

In addition, in six out of nine subjects a seminal plug was observed. In five animals, the ejaculate appeared during the very first cluster, that is, during the first minute of the test session.

Table 1 Penile responses after saline and muscarine application

	Saline	*Muscarine*
Number of clusters	12.3 ± 3.8	18.6 ± 3.9**
Erections per cluster	4 ± 1	5.3 ± 1.5
Penile cups per cluster	1.6 ± 1.3	3.2 ± 1.2*
Flips per cluster	1.3 ± 1	0.9 ± 1
Long flips per cluster	1.4 ± 1.33	0
Ejaculation	Zero out of nine	Six out of nine

Data are shown in terms of mean ± s.d., except for ejaculation ($N=9$).
$*P<0.001$; $**P<0.01$, Newman–Keuls test.

The remaining animal ejaculated twice during the 20 min session, once 6 min after the start of the test during the fifth cluster, and the second time at the 18 min, during the 20th cluster. None of the animal ejaculated after saline. Plugs were withdrawn at the end of the test and observed under the microscope. Spermatozoa were found in every single plug.

Discussion

The penile responses found in the present experiments after saline application agree well with those reported previously in spinal cord-transected subjects.[9,13] Muscarine produced a facilitation of PR as revealed by the significant increase in the number of penile response clusters, erections and penile cups. The number of erections within the cluster of the subjects of the M group was not different from those of the S group (Table 1), thus the increase in the number of erections in response to muscarine is due to the increase in the number of clusters. The number of the cups within the clusters was higher in the M group than those of the S group. Therefore, the increase in the number of penile cups after muscarine is produced both by the increase in the number of clusters as well as the increase of the number of cups within the cluster. In addition, muscarine also increased significantly the duration of the penile cups (Figure 1b and d). Thus, erections and penile cups are influenced differently by the drug. At the present, we have no explanation for this finding.

Since the first description of the method for eliciting reflexive erections[9] and in most reports thereafter, it has been reported that ejaculation occurs very rarely.[19,20] Thus, one of the most striking findings of present results is that 66% of rats receiving muscarine ejaculated during the test. Owing to the systemic application of the drug, it cannot be discarded that muscarine may produce ejaculation or facilitate glans erections by a direct effect in peripheral tissues, that is, by eliciting contractions of seminal vesicles without neural mediation or by the blood filling of the corpus cavernosum, since the stimulation of the muscarinic receptors produces contractions of the seminal vesicles,[21–23] and penile erection is a parasympathetic-mediated response.[8]

However, it has been shown previously that ejaculation is facilitated despite the subjects being treated with a peripheral parasympathetic inhibitor before the systemic application of physostigmine, suggesting that the facilitation was mediated centrally.[2] Moreover, intrathecal application of neostigmine facilitates erection and ejaculation,[1] and also intrathecal administration of muscarine facilitates sexual behavior in freely moving rats.[3] Thus, in view of the present results, it can be speculated that the facilitation of ejaculation is mediated by the stimulation of central muscarinic receptors.

Further support to this interpretation is provided by the facilitation of PR by muscarine, since PR are generated by an intrinsic spinal pacemaker.[24] Besides, not only does the filling of the corpora cavernosa produces the penile cups but also the contraction of BS.[24] Since the activity of BS muscles cannot be produced directly by muscarine, a central action of muscarine is also supported. Furthermore, a propriospinal cholinergic pathway has been described,[25] and muscarinic cholinergic receptors have been found at the dorsal horn and in motoneurons.[26–28] In addition, the cholinergic neurons of the spinal cord are interconnected widely in longitudinal and transverse bundles,[29–31] which are consistent with an organization of a common function.[29]

It is also worth noting that the penile long flips were inhibited by muscarine. Even though penile flips occur mostly without glans erections[9,17,19] and are produced by the contraction of the ischiocavernosus muscles,[17] it is hard to explain the differential effect produced by muscarine on penile flips and long flips. Nonetheless, this result is suggestive also of a central effect produced by muscarine on the spinal pacemaker for PR.

Taken together, earlier,[1–3,5] and present results suggest that spinal cord M-type cholinergic receptors may be involved in both, erectile and ejaculatory mechanisms.

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