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Muscarinic mediation of the urethro genital reflex in spinal cord-transected rats

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Abstract

The effects of muscarinic receptor stimulation were tested on the urethro genital reflex (UGR) in anesthetized and spinal cord-transected rats. Drugs were applied directly to the spinal cord. The electromyographic activity (EMG) of the bulbospongiosus (BS) muscle was used for recording UGR. In six animals BS as well as soleus, posterior biceps or peroneus tertius muscle EMG was recorded simultaneously. Muscarine (5, 10, 20, 50 and 100 μg) was applied in 22 animals after cutting L6–S1 dorsal roots. Some observations were made on another six animals, to which an extensive bilateral dorsal rhizotomy (L3–S2) was performed. Rhythmic bursts of similar frequency and size to those seen during UGR were found in BS muscle a few minutes after muscarine application. No rhythmic bursting was found on the hindlimb muscles, but exclusively on BS muscles. The effects of homatropine (25, 50, 100 and 200 μg), an acetylcholine muscarinic receptor antagonist, were tested in 21 rats after UGR was elicited three times at low stimulation intensity (7 mm Hg). Homatropine produced two effects: (i) A significant increase in the latency of UGR. (ii) A facilitation of UGR inhibition. In view of these results it can be speculated that muscarinic receptor stimulation is involved in the elicitation of UGR.

Keywords: Muscarine; Atropine; Sexual reflexes; Ejaculation; Urethro genital reflex

1. Introduction

The neurochemical control of masculine sexual behavior has been the subject of several studies (for review see Ref. [40]). Despite these efforts, the topic is still elusive, and controversial results have been reported for every substance tested [8,12,40,42,49]. The great variety of receptor subtypes for the drugs used, and the different neural structures involved in the control of sexual behavior, can account for these results.

In normal copulation, the inflow from the urethra seems to play no important role for the male rat to ejaculate [7,24,30,38,48]. The mechanical stimulation of the pelvic urethra, however, has been used to elicit the urethro genital reflex (UGR) in spinal cord-transected rats. UGR is an experimental model of sexual climax in both male and female rats [15,39]. In the male rat, UGR is characterized by penile erection, emission of the urethral contents, and rhythmic activity on the perineal muscles and on the sympathetic and parasympathetic efferents [15,39].

UGR has permitted the identification of the nucleus paragigantocellularis (PGC) as the source of sexual reflex inhibition [32], and the role of 5-hydroxytryptamine (5-HT) in this reflex inhibition [33,34]. Chronic lesions of PGC have been shown to increase copulatory efficiency. Furthermore, the UGR presents some characteristics found in normal copulation, such as facilitation and exhaustion [20].

Besides the inhibitory role of 5-HT, the neurotransmitters involved in the control of UGR have not as yet been identified. A better understanding of the mechanisms involved in sexual climax may be provided if the chemical signals controlling the UGR are known. This may be of importance to the knowledge of the male sexual behavior, because little is known of the spinal neurochemical control and of the spinal mechanisms that control the ejaculation [40].

Systemic application of muscarinic receptor agonists and antagonists reduced copulatory efficiency in rats [11,31,46] and rabbits [1]. Differential effects were found, however, if

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the drugs were injected into the medial preoptic area: the agonists increased and the antagonists decreased copulatory efficiency [26,27]. Also, it has been suggested that acetylcholine may play a role in the phenomenon of premature ejaculation [2]. In addition, it was described recently that muscarine may induce locomotor-like patterns of activity on neonatal rat spinal cord [16,17] as well as rhythmic activity of lumbar motoneurons of a slice preparation from adult turtles (Höunsgaard J, personal communication) and in brain slices of guinea pigs [13]. Thus, it would be of interest to test whether cholinergic agonists or antagonists may influence the performance of UGR. This is the goal of the present experiments.

2. Materials and methods

The principles of laboratory animal care, as well as the local requirements of the ethics committee of our institutions, were followed.

2.1. Experimental animals

Male Wistar rats (250–380 g) from our colony were used. The animals were kept in a reversed light cycle (lights on: 22:30 h, off: 10:30 h) with a room temperature of 21°C. Three animals were housed per cage. Food and water were supplied ad libitum. All animals that were used in the present experiments were sexually inexperienced.

2.2. Surgical procedures

2.2.1. General procedures

The rats were initially anesthetized with urethane (1.6 g/kg, i.p.). The trachea was cannulated to prevent aspiration of saliva. To locate the UGR spinal generator the spinal cord was transected at different levels from T10 to S1 in eight animals. Only one spinal cord segment was transected in every animal. In 43 rats the spinal cord was transected at the T6 spinal segment, according to the anatomical schema of Hebel and Stromberg [23]. Gelfoam was inserted into the spinal canal to prevent bleeding. A lumbosacral laminectomy (L3–S2) also was performed. The dura was cut and the lumbosacral spinal cord was exposed. A pair of platinum needle electrodes (Grass E2, W. Warwick, RI) was inserted in the bulbospongiosus (BS) muscle. At the end of the experimental observations an overdose of pentobarbital was applied to all animals.

Before the lumbosacral laminectomy was done, in the homatropine-treated rats [21] the pelvic organs were exposed by a single midline incision. A PE-50 (0.965 mm o.d.) catheter was inserted into the pelvic urethra via a bladder incision. The catheter was firmly tied to the bladder neck and the wound was closed. The urethral catheter was connected to a syringe pump attached by a three-valve stopcock to a pressure transducer.

The pelvic urethra was not catheterized in the muscarine-treated animals. Instead a bilateral (L6–S1) dorsal rhyzotomy was performed at the end of the lumbosacral laminectomy. In another six animals an extensive dorsal rhyzotomy (L3–S2) was made. In these animals needle electrodes were inserted in the BS as well as in the Sol, PB and/or Per muscles. In these six rats the femoral artery was dissected free from the surrounding tissues. A catheter, filled with heparin solution (100 U/ml), was introduced into the artery for monitoring blood pressure. The dorsal roots were left intact in the homatropine-treated rats.

2.3. Stimulation and recording

As previously reported [15,20,39], electromyographic (EMG) activity of the BS muscles was used for monitoring the UGR. EMG activity was recorded on a polygraph using conventional techniques in all animals. In the six animals with L3–S2 dorsal rhyzotomy the recordings were digitized through an A/D converter (Axon DigiData Interface, Axon Instruments, Foster City, CA) and stored on a PC computer for later analysis.

2.3.1. Homatropine

Two hours after spinal cord-transection UGR was evoked in all animals. After three consecutive successful attempts with low stimulation intensity (7 mm Hg), homatropine (25, 50, 100 and 200 μg), an acetylcholine muscarinic receptor antagonist, was topically applied to the spinal cord. A small piece of cotton soaked in the homatropine solution was left covering the dorsal cord. Twenty minutes after homatropine application, tests were done to determine whether the stimulation of the pelvic urethra could still induce the UGR. UGR was evoked as reported previously [20]. Briefly, a pressure increase on the urethra was produced by injecting saline solution (200 μl/min, Harvard Syringe Pump, Harvard Apparatus, South Natick, MA) through the catheter while occluding the urethral meatus. Different pressure intensities (7–120 mm Hg) were used. Pressure changes were made by using different injection times (3 up to 90 s). Every intensity was tested at least three times with an interval of 3 min between trials. The urethral pressure changes were measured with a pressure transducer that was connected to a polygraph (Grass M7).

2.3.2. Muscarine

To test the effects of muscarine, an acetylcholine receptor agonist, 2 h after spinal cord-transection the drug (5, 10, 20, 50 and 100 μg) was topically applied to the spinal cord. A small piece of cotton soaked in the muscarine solution was left covering the dorsal cord. EMG activity of the BS muscles was recorded continuously after application of muscarine to the spinal cord. In those six animals in which an extensive dorsal rhyzotomy was done, 100 μg of muscarine were applied, and the

EMG activity of BS as well as Sol, pB and/or Per hindlimb muscles was recorded continuously.

2.4. Drugs

Muscarine [(+)-muscarine chloride, M6532] and homatropine (DL-Homatropine hydrobromide, H0126) were purchased from Sigma (St. Louis, MO). Both drugs were diluted in saline solution to reach the final concentration.

2.5. Data analysis

The effects of homatropine were analyzed by the following parameters: (1) number of successful trials, (2) latency of UGR and (3) frequency of rhythmic bursts. Only the burst frequency was examined for the muscarinic effects. Two-way ANOVA test was used for statistical analysis. A value of $p < 0.05$ was considered statistically significant.

3. Results

The location of the UGR spinal generator was searched before examining the effects of the drugs. The spinal cord was transected at different levels from T10 to S1. Only one spinal cord segment was transected in every animal. UGR could still be induced in T10–L1 spinal cord-transected animals. In none of the animals was UGR evoked if the spinal cord was transected lower than the L2 segment. The L2 anatomical segment corresponds to lumbar enlargement [23]. Thus, drugs were applied to the L2–L5 anatomical segments.

3.1. Effects of muscarine

A few minutes after the application of muscarine, 81% (18 out of 22) of the animals showed rhythmic bursting in the BS muscle (Fig. 1A–C), independently of the dose of agonist applied. All the animals (10 out of 10) that received 5, 10 and 100 μg showed rhythmic activity. Five out of seven and three out of five animals receiving 20 and 50 μg, respectively, also showed the rhythmic bursting (Table 1).

Except for the animals receiving 5 μg, BS muscle activity was similar to that found during UGR. No significant difference was found in the burst frequency after muscarine application compared to the burst frequency that is present during UGR (Table 1). Because the rhythmic bursting of the BS muscle was similar to that found in the reflex, it was called "drug-induced UGR." These drug-induced UGRs can clearly be counted as episodes: after the rhythmic activity was present in the BS muscle, the EMG was silent for several minutes and a new episode of rhythmic bursting appeared. The BS activity was considered a drug-induced UGR episode if it has at least three bursts with a frequency similar to that of UGR.

As can be seen in Fig. 1C, these drug-induced UGR episodes also included "post-discharges" that have been

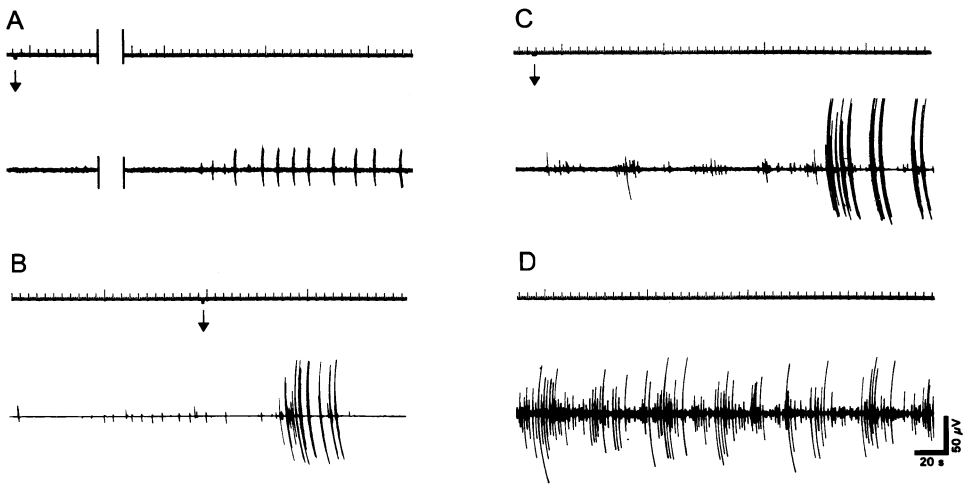

Fig. 1. Drug-induced UGR episodes induced by different doses of muscarine. In (A–C), the rhythmic bursts of the BS muscle after muscarine (arrow) application are shown. A total of 5 μg were applied in (A), 50 μg in (B) and 100 μg in (C). Though 5 μg elicited rhythmic bursting, its frequency was different from that found in UGR. BS EMG activity shown in (B) and (C) was entirely similar to that found in UGR. Muscarine application is marked by the arrow. The results are taken from three different animals. Ten minute latency was found for the bursting activity evoked by 5 μg. The vertical bars in (A) indicate that the traces were cut to show the response. In (D), sustained activity of the BS muscle that appeared 2 min after the last UGR episode of the same animal shown in (C) is also shown. It can be seen from the EMG record that the burst frequency is similar to that of the UGR reflex and also that few muscular units are recruited during the sustained activity. Top trace shows the time marking and bottom trace the EMG activity of the BS muscle in all records. The same amplification was used in all EMG recordings.

Table 1
Effects of muscarine application on the spinal cord

Dose	Control	5 μg ($n=3$)	10 μg ($n=4$)	20 μg ($n=7$)	50 μg ($n=5$)	100 μg ($n=3$)
Number of animals responding[a]	10 out of 10	3 out of 3	4 out of 4	5 out of 7	3 out of 5	3 out of 3
Latency[a] (min)	–	10.5 ± 3.5	3.15 ± 0.75	7.8 ± 1.5	3.4 ± 0.8	2.27 ± 0.8
Frequency[a] (Hz)	0.8 ± 0.05	4.46 ± 1.19 *	0.73 ± 0.1	0.80 ± 0.09	0.97 ± 0.08	1.17 ± 0.1
Number of spontaneous UGRs[a]	–	4 ± 2	5.6 ± 2.86	5.6 ± 1.01	5.5 ± 1.5	5 ± 2
Sustained oscillation[b]	–	1	4	2	1	2
Frequency[b] (Hz)	–	3.72 ± 1.1 *	0.92 ± 0.05	0.60 ± 0.1	0.97 ± 0.05	1.17 ± 0.1
Duration[b] (min)	–	225	225	225	225	225

* $F_{1,2}=3.12$; $p<0.01$ (two-way ANOVA test).

[a] Data of the rhythmic bursts immediately after muscarine application. Latency of the first trend of bursts after muscarine application. Frequency and number of bursts are expressed as mean and standard error of the mean. Number of spontaneous UGRs refers to the number of times that the spontaneous oscillation resembled the UGR appeared.

[b] Data from the animals that showed sustained oscillations after muscarine. Number of rats showing sustained activity in every dose tested. Frequency is expressed as mean and standard error of the mean. All experiments were concluded after 4 h of observation.

described previously [20]. Furthermore, the drug-induced UGR episodes appeared several times (two to eight times; Table 1) in all animals. The latency between the episodes lasted several minutes (1–3 min) in all doses used. It is important to note that the effects produced by muscarine were found despite the fact that the pelvic urethra was not stimulated and L6–S1 or L3–S2 dorsal roots were cut bilaterally.

In three other animals (20, 50 and 100 μg) the urethra was cannulated to examine whether the drug-induced bursting of the BS muscle was in fact a UGR. Penile erection as well as an increase in the urethral pressure were found during the drug-induced bursts of the BS muscle.

Muscarine at a dose of 5 μg also evoked bursts of activity in the BS muscle. The burst frequency, however, was different from the burst frequency that is found in UGR (Fig. 1A, Table 1). The activity of the BS muscle that was elicited by this dose was not considered to be a drug-induced UGR.

After muscarine application, 45% (10 out of 22) of the animals showed sustained bursts of activity in the BS muscle (Table 1). Except for the sustained activity

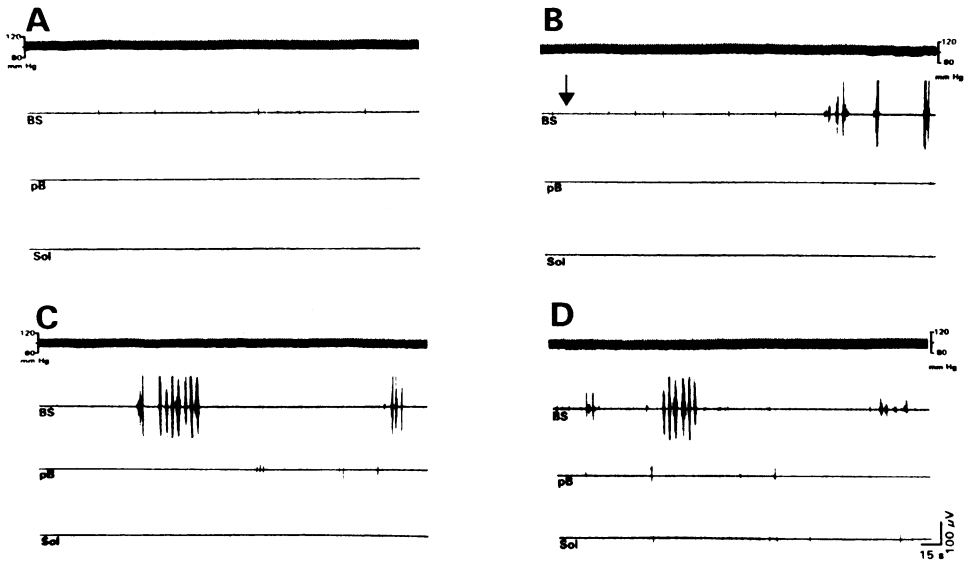

Fig. 2. Muscarinic elicitation of drug-induced UGR rhythmic bursts. (A–D) Continuous recordings of blood pressure and EMG activity of several muscles in one experiment. (A) Recordings during the control period before the application of muscarine. As shown, no EMG activity was present on pB or Sol muscles. Few units of BS muscle were firing. In (B), 100 μg of muscarine were applied to the spinal cord (arrow). Approximately 3 min after drug application rhythmic bursts appeared in the BS muscle. As shown, these rhythmic bursts resembled those found in the UGR reflex. The other two drug-induced UGR episodes appeared during the recording period (C,D). No change in blood pressure was found in this experiment. Also, no rhythmic activity was found on hindlimb muscles, even though some units of both muscles were firing during the observed period. Top beam shows the blood pressure recording, second beam BS muscle, third beam pB muscle and last beam Sol muscle EMG activities. The calibration shown is the same for all figures.

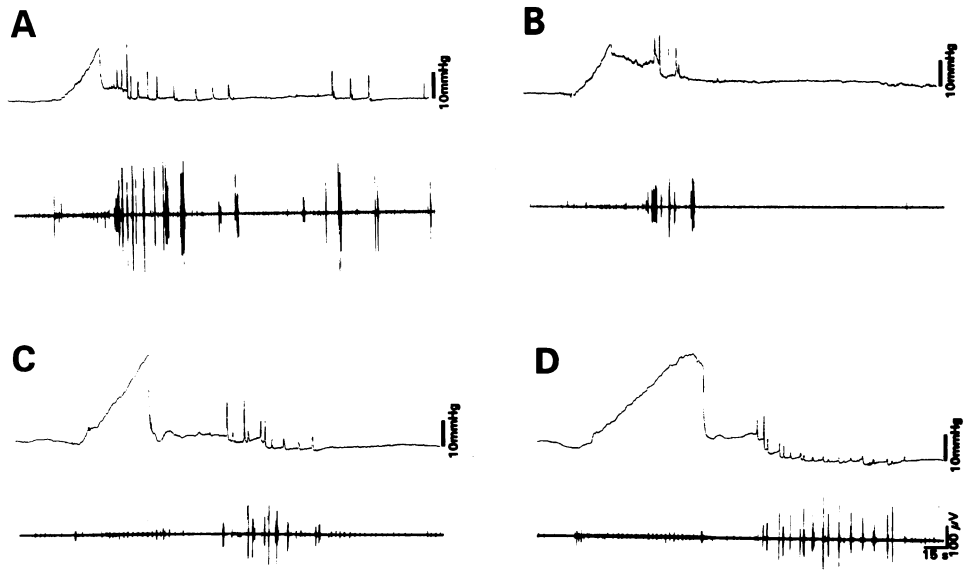

Fig. 3. Latency increase of the UGR reflex produced by homatropine. (A) Control UGR: Before drug application the reflex was induced by increasing pressure (7 mm Hg) in the pelvic urethra. (B–D) show the UGR response after 25 μg of homatropine. All records are from the same animal. As can be seen, there is a latency increase of the reflex in (B–D). Also, few units are recruited after homatropine. UGR was successfully elicited after two failures in (B) (7 mm Hg), (C) (30 mm Hg) and on the first attempt in (D) (120 mm Hg). Same amplification was used for all the records. Top trace shows the pressure recording and bottom trace the EMG activity of the BS muscle.

that was elicited by 5 μg, the burst frequency was not different from that found during UGR. As can be seen from the EMG records (Fig. 1D), fewer muscle units were recruited in the bursts of sustained activity, compared to the number of muscle units that were recruited in the bursts of the drug-induced or the actual UGR. In addition, no penile erections were found during this sustained activity. In all animals showing this sustained activity, it continued until the end of the experiment (less than 4 h).

All six additional animals with extensive dorsal rhizotomy also showed the rhythmic bursting in the BS muscle. Interestingly, no rhythmic bursting was found in the hindlimb muscles (Fig. 2). It is important to notice also that muscarine did not elicit locomotor-like activity, micturition or defecation in any of the observed animals. No change was found in the arterial pressure after the agonist application in five out of the six animals (Fig. 2). A slight decrease of the arterial pressure and a significant decrease of the heart rate was found in the last animal.

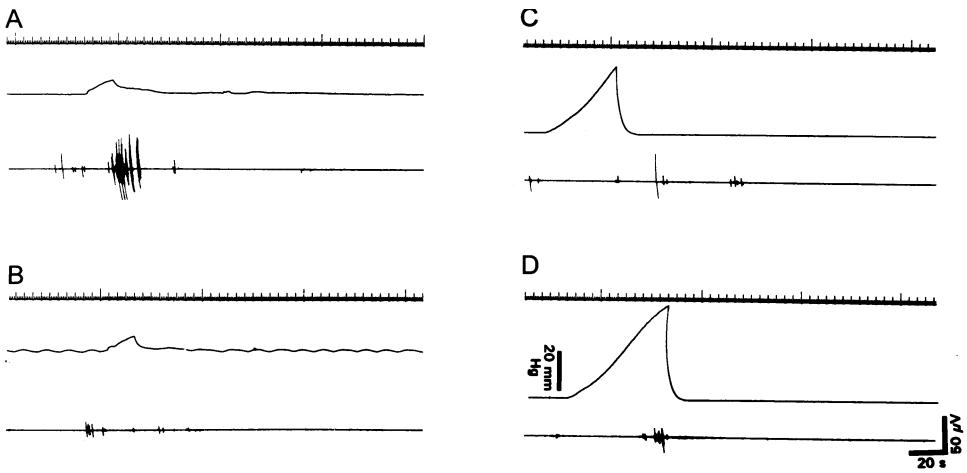

Fig. 4. Complete inhibition of the UGR reflex after homatropine. (A) Before drug application UGR was elicited by a pressure increase of 7 mm Hg on the urethra. As shown, the typical bursts of the BS muscle appeared immediately at the end of the stimulation. (B–D) UGR could not be evoked at all after 100 μg of homatropine at any stimulation intensity. As shown, however (D), a slight response was seen in the BS muscle during the stimulation period. Three successive failures were found for 7 (B), 70 (C) and 120 mm Hg (D). Top trace shows the time marking, middle trace the pressure recording and bottom trace the EMG activity of BS muscle.

Table 2
Effects of homatropine application on the UGR

Dose	Control	Intensity	25 μg ($n=5$)	50 μg ($n=5$)	100 μg ($n=4$)	200 μg ($n=7$)
Latency	1.93±0.5 s	7 mm Hg	8.2±2.1 s*	26.57±6.8 s**	6±16 s*	19±4.14 s**
	1.63±0.4 s	30 mm Hg	14.6±4.2 s*	15.6±6.3 s**	8.2±2.3 s*	6.2±1.6 s**
	1.84±0.5 s	70 mm Hg	27.0±15.5 s*	24.5±8.6 s**	3.6±1.6 s*	57.7±21.8 s**
	1.83±0.4 s	120 mm Hg	9.0±6.3 s*	16.0±5.6 s**	10.8±3.6 s*	39.0±15.9 s**
Frequency (Hz)	0.8±0.05		0.84±0.09	0.77±0.08	0.93±0.1	0.94±0.05
Success/No Success	1		0.46	0.36	0.43	0.47

Data of latency and frequency are expressed as the mean and standard error of the mean. The burst frequency was not statistically different for the different stimulation intensities. Success/No Success = number of trials in which the stimulation evoked UGR/number of trials in which the stimulation did not elicit UGR.

* $p<0.03$ (two-way ANOVA).

** $p<0.01$ (two-way ANOVA).

3.2. Effects of homatropine

The effects of homatropine, an acetylcholine muscarinic receptor antagonist, were examined to further test whether muscarinic receptors are involved in the elicitation of UGR. Before the application of the drug, the pelvic urethra was stimulated three times with an interval of 3 min. Low stimulation intensity (7 mm Hg) was used to stimulate the urethra. In all animals the reflex was elicited in every attempt (see for example Figs. 3A and 4A). No further stimulation was applied to avoid the inhibition of the reflex [20]. Homatropine was applied to the spinal cord after the control stimulation.

UGR was still evoked with low stimulation intensity after homatropine in 80% (17 out of 21) of the animals. In most animals, however, UGR was not elicited at the first trial, and it did not occur until the second or the third attempt. UGR could be evoked again but, after one or two successful trials, it could no longer be induced. In addition, when it was evoked, the latency of the reflex was significantly longer (25 μg, $F_{4,39}=1.31$; $p<0.03$; 50 μg, $F_{4,46}=3.19$; $p<0.01$; 100 μg, $F_{4,38}=1.67$; $p<0.03$; 200 μg, $F_{4,45}=3.52$; $p<0.01$) than that of the control trials. Also, the reflex was inhibited after a few trials (it was considered that the reflex was inhibited when UGR could not be evoked with the same stimulation intensity for three consecutive trials; see Ref. [20]). As is stated in Table 2, UGR was elicited in less than 50% of the trials. Similar results to those described above were found for every dose examined (Table 2).

It has been described that if the reflex is elicited no longer at a certain stimulation intensity, it could be evoked again by increasing the intensity. This pattern was present for all stimulation intensities that were examined [20]. Thus, it was tested whether homatropine interfered with this UGR property. As can be seen in Fig. 3C–D, increasing the stimulation intensity would in fact elicit the reflex in each of the doses used (Table 2).

A difference was found, however, after drug treatment. In the control animals the UGR latency at the new stimulation intensity level was always shorter than the last one [20]. This was not found after homatropine: the latency of the first evoked UGR was the same or longer than the last UGR. As it was found at the lowest stimulation intensity, homatropine increased the number of failures. UGR was elicited less than 50% of the time, at every stimulation intensity (Table 2).

In addition, in four animals UGR could not be evoked after homatropine application (Fig. 4). This result was found at the highest doses of the drug (two rats for 100 μg and two rats for 200 μg).

4. Discussion

A central pattern generator for UGR has been suggested [39]. In our experiments the UGR could be evoked if the spinal cord-transection was done above the L2 segment. In no rat UGR could be evoked if the transection was at or below the L2 anatomical segment. Thus, it can be suggested that the pattern generator may be located below this segment. This spinal cord anatomical level corresponds to the lumbar enlargement [23]. The L5–S1 spinal cord segments have been suggested as the location of the spinal interneurons that are related to sexual function [25,35,36,44]. Thus, the drug application was circumscribed to the lumbar enlargement.

It was found in the present experiments that cholinergic agonist muscarine (10–100 μg) application to the spinal cord produced "drug-induced UGR" episodes in 68% of the animals. As pointed out in the Results section, these episodes of burst activity in the BS muscles were considered to be "drug-induced UGR" for the following reasons: (i) Both penile erection and an increase in the urethral pressure were found during BS muscle bursts. (ii) No statistical difference was found between the burst frequency of drug-induced UGR episodes compared to that of real UGR. (iii) As it was found in real UGR, drug-induced UGR episodes also showed post-discharges. (iv) Finally, these episodes of muscle activity were spread over several min-

utes. Thus, it may be concluded that the UGR central pattern generator may be activated by the stimulation of muscarinic receptors. The fact that L6–S1 (or L3–S2) dorsal roots were cut and the urethra was not cannulated may also support this interpretation.

In addition, 45% of the animals also showed sustained activity in the BS muscle. This sustained activity in the muscle was elicited in each of the doses tested (Table 1). In all animals in which this sustained activity was present, it appeared 6–10 min after the UGR episodes and continued until the end of the experiment. Despite similarity of the burst frequency to that of UGR, no penile erection was found and few muscular units were recruited during this sustained activity. Thus, it is difficult to consider it to be a typical and sustained UGR, but it may reflect a sustained activity of the UGR central pattern generator that was activated by the cholinomimetic agent. No rhythmic activity was present in the hindlimb muscles during the periods of sustained activity on BS muscle. Thus, no other central pattern generator was stimulated by muscarine.

Further support for the involvement of muscarinic receptors in UGR generation is given by the effects that were produced by homatropine. Although UGR was inhibited completely by homatropine in only 19% of the animals (Fig. 4), an inhibitory effect of the drug was revealed in the rest of the animals treated: UGR behaved as though it was near the point of inhibition after being elicited in several trials. As shown in Fig. 3 and Table 2, a significant latency increase and a great number of failures were found after homatropine.

UGR was elicited three times before homatropine was applied because it was necessary to show that UGR was present and was necessary in order to measure its latency. Thus, it can be argued that the latency increase was the result of the inhibition produced by repeated stimulation instead of being an effect of the drug. If, however, the previous stimulation had had an effect on the subsequent ones, this effect should be a decrease instead of an increase in the latency [20]. To the contrary, UGR could not be elicited on the first attempt after drug application in most animals, independently of the dose used. Furthermore, homatropine increased the number of failures. Thus, it may be concluded that the changes of UGR were produced by the drug and not by the previous stimulation.

It is hard to determine the muscarine site of action. First of all, as was pointed out earlier [28], the method employed is totally unphysiological because, on the one hand, the spinal neurons are subjected to a sustained exposure of the agonist and, on the other hand, the agonist will stimulate all neurons that have receptors for it, regardless of whether the neurons are or not blocks of the central pattern generator. The fact still remains, however, that the stimulation of muscarinic receptors induces a rhythmic bursting on BS muscles that is similar to that found in UGR.

One major question is whether the drug produces a non-specific increase in the excitability of the spinal cord. If this is so, it may be that the stimulation of muscarinic receptors also produces the activation of other spinal cord generators (i.e., scratching, locomotion, micturition, defecation, and so on). For example, muscarine application to the isolated spinal cord of the neonatal rat produces locomotor-like activity [16,17]. Although this question is well beyond the scope of the present work, in the present experiments muscarine did not elicit micturition, defecation or locomotor-like movements in a single animal. As shown in Fig. 2B–D, whereas muscarine produced rhythmic activity on the BS muscles, it did not induce rhythmic bursting on the Sol or the pB muscles. These findings did not exclude a subthreshold activation of other spinal cord generators. However, it has been reported previously that locomotion or micturition cannot be evoked in acute spinal preparations [3,4,6,18,29]. Furthermore, we have found that the copulatory efficiency of normal male rats is affected by the intrathecal application of muscarine or homatropine to the lumbar spinal cord. The copulatory efficiency is increased by the agonist and it is decreased by the antagonist. No change on locomotion was found in these experiments (unpublished observations).

The assumption of a specific effect of the cholinomimetic agent on UGR can also be supported by the lack of effect of muscarine on arterial pressure or heart rate that was found in five out of six animals. A slight decrease of both heart rate and arterial pressure was found in one of the animals. The decrease of arterial pressure produced by intrathecal muscarine has been reported previously [10]. In addition, cholinesterase inhibitors, applied intrathecally or systemically, have been reported to facilitate ejaculation in spinal cord-injured men ([14,22], see also Ref. [49] for review). The fact that muscarine increases copulatory efficiency also shows that both in copula and ex copula ejaculatory patterns respond similarly to drug treatment. This may be of importance to the study of the spinal neurochemical control of ejaculation.

The range of doses examined were 10–100 μg (μM range). Thus, it can be argued that high doses of drugs were used. Similar or higher doses than these have been used by others to test the effects of drugs on the spinal cord [5,9,21,37,45,47] in the brain [43] or in in vitro preparations [19,41].

In view of the present results, it may be concluded that stimulation of muscarinic receptors is involved in the generation of the UGR.

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