Central Muscarinic Mechanisms Regulating Voiding in Rats

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ABSTRACT

The influence of muscarinic receptor stimulation and blockade on the central regulation of micturition was evaluated in conscious female rats. Saline was infused into the bladder to induce repeated bladder contractions and voiding. Increasing doses of a muscarinic agonist, oxotremorine-M (OXO-M; 0.01 to 1 μg/rat) or antagonist, atropine (0.1 to 30 μg/rat) were administered. Intrathecal OXO-M (0.1 μg) increased bladder capacity (BC; 85 ± 17%), but did not change maximal voiding pressure (MVP), pressure threshold (PT), postvoiding intravesical pressure, or voiding efficiency (VE). Intracerebroventricular OXO-M (0.1 μg) increased BC (97 ± 6%), MVP (45 ± 19%), PT (158 ± 49%), and reduced VE (−17 ± 5%). A larger dose of OXO-M (1 μg, either i.c.v. or i.t.) produced greater changes. These effects were not reproduced by i.v. injections of OXO-M. The effects of OXO-M were blocked by pretreatment with atropine in a dose (1 μg i.c.v. or i.t.), which alone had no effect on voiding parameters. A larger dose of atropine (10 μg) reduced BC (−31 ± 7% i.c.v. and −34 ± 6% i.t.) and VE (−21 ± 3% i.c.v. and −25 ± 5% i.t.) but increased BC (52 ± 8% i.c.v.). These results indicate that activation of muscarinic receptors in the brain or spinal cord can suppress voluntary voiding, but also stimulates bladder activity during bladder filling. The muscarinic inhibitory mechanisms do not appear to be tonically active. The effects of atropine (i.c.v. and i.t.) indicate that muscarinic excitatory mechanisms are tonically active. These findings raise the possibility that voiding function is regulated by both inhibitory and excitatory cholinergic mechanisms in the central nervous system.

Muscarinic and anti-muscarinic agents have been used clinically for many years to treat disorders of the lower urinary tract (Chapple, 2000). Although the effects of these agents are usually attributed to their action on peripheral muscarinic receptors, it is also possible that they have effects on central muscarinic receptors located in the brain and spinal cord (Li et al., 1991; Zhou and Gebhart, 1991; Bartolini et al., 1992; Aagaard and McKinney, 1993; Iwamoto et al., 1993; McKinney et al., 1993).

Previous studies in animals revealed excitatory and inhibitory effects of cholinergic drugs on central neural pathways controlling lower urinary tract function. In decerebrate cats, injection of the cholinergic agonist, carbachol, into the region of the locus coeruleus alpha (the pontine micturition center) evoked bladder contractions and voiding (Sugaya et al., 1987). This effect was blocked by atropine, indicating that it was mediated by activation of muscarinic receptors. Administration of the muscarinic agonist, bethanechol, to the brain of anesthetized dogs via a vascular cross-perfusion technique from a donor dog, reduced bladder capacity and increased maximal voiding pressure (O’Donnell, 1990). In rats anesthetized with pentobarbital, the bladder hyperactivity induced by l-DOPA seems to be mediated in part by activation of central muscarinic receptors, because it was reduced by atropine, which acts at both central and peripheral muscarinic receptors, but not by methylscopolamine, a quaternary muscarinic antagonist that does not penetrate the blood-brain barrier (BBB) (Sillen et al., 1982). Intravenous administration of oxotremorine, a muscarinic agonist that enters the brain, facilitated the L-DOPA-induced bladder hyperactivity. This effect was not blocked by methylscopolamine, indicating a central site of action. Thus studies in several species indicate that voiding can be facilitated by central muscarinic mechanisms.

Other studies identified central inhibitory mechanisms. Injections of carbachol into brain stem sites ventral to the pontine micturition center (Matsuzaki, 1990) or into the locus coeruleus (Roppolo et al., 1987) suppressed bladder activity and increased bladder capacity in the decerebrate cat. These effects were blocked by microinjections of atropine into the pons. However, administration of atropine alone did not alter bladder activity, suggesting that muscarinic mechanisms in the pons do not have a tonic influence on voiding function in the decerebrate animal (Roppolo et al., 1987). Evidence for muscarinic inhibitory receptors has also been obtained in the rat (Thor et al., 2000). Intravenous administration of oxotremorine, a muscarinic agonist, or physostigmine, an anticholinesterase agent, suppressed reflex contrac-

ABBREVIATIONS: OXO-M, oxotremorine methiodide; CMG, cystometrogram; BC, bladder capacity; VV, voided volume; RV, residual volume; MVP, maximal voiding pressure; PT, pressure threshold; PVIP, postvoiding intravesical pressure; VE, voiding efficiency; CNS, central nervous system; PMC, pontine micturition center; PLSD, protected least significant difference; BBB, blood-brain barrier; ANOVA, analysis of variance.
tions of the anal sphincter induced by nociceptive stimulation of the urinary bladder of anesthetized rats. The effects were blocked by atropine but not by a peripherally acting antimuscarinic agent. These studies raise the possibility that inhibitory muscarinic mechanisms control the central processing of afferent input from the bladder.

The contribution of cholinergic transmission to the central regulation of micturition was evaluated further in the present study. We examined the changes in voiding function in awake normal rats following i.c.v., i.t. or i.v. administration of a muscarinic agonist (oxotremorine methiodide, OXO-M) or antagonist (atropine). A preliminary account of this work has been presented in an abstract (Ishiura et al., 1999).

**Materials and Methods**

**Animal Preparation.** Female Sprague-Dawley rats weighing 250 to 300 g were anesthetized with halothane (2% in oxygen) for surgical insertion of either an i.c.v., i.t. or i.v. catheter and an intravesical catheter. To implant an i.c.v. catheter, the rats were positioned in a stereotaxic frame, a scalp incision was made over the sagittal suture, and a hole (diameter approximately 1.0 mm) was drilled in the right parietal bone to expose the dural surface 1.0 mm lateral and 0.3 mm anterior to the bregma. A sterile stainless steel catheter (o.d. 0.6 mm, i.d. 0.3 mm, length 10.5 mm) was lowered 5.3 mm from the bregma with a micromanipulator. With the aid of a small screw placed in the skull as an anchor, the catheter was fixed to the skull with dental acrylic. Solutions were injected via an infusion catheter (o.d. 0.3 mm, i.d. 0.1 mm) inserted into the larger catheter. Single doses of drugs were administered in a volume of 1 ml and the infusion catheter was left in place for 1 min after infusion to allow for diffusion of the drug solution. At the end of the experiment, Blue Food Color (McCormick, Hunt Valley, MD) was injected to verify the location of the cannula tip.

An i.t. catheter was inserted with a technique developed by Yaksh and Rudy (1976). The occipital crest of the skull was exposed and the atlanto-occipital membrane was incised at the midline with the tip of an 18-gauge needle. A catheter (PE-10) was inserted through the slit and passed caudally to the L6 level of the spinal cord. The volume of fluid within the cannula was kept constant at 9 µl in all animals. Single 1-µl volumes of drug solutions were administered followed by a 10-µl flush of artificial cerebrospinal fluid (Feldberg and Fleischhauer, 1960). At the end of the experiment, a laminectomy was performed to verify the location of the cannula tip. For i.v. drug administration, a catheter (PE-50) was placed in the left jugular vein. Single 100-µl volumes of drug solutions were administered in sterile physiological saline (0.9% sodium chloride solution) followed by a 100-µl flush of sterile physiological saline.

The urinary bladder was catheterized using the method of Yaksh et al. (1986). The bladder was exposed via a midline abdominal incision. A catheter (PE-50), the bladder end of which was heated to create a collar, was inserted through a small incision at the bladder dome, and a suture was tightened around the collar. The other end of the catheter was passed through subcutaneous tissue and exited through the skin. After closing the abdominal incision by suturing the muscle and skin, the rats were placed in a restraining cage that was large enough to permit them to adopt a normal crouching posture, but narrow enough to prevent them from turning around. The rats were subsequently allowed to recover from halothane anesthesia.

The bladder catheter was connected via a T-stopcock to a pump for continuous infusion of physiological saline and to a pressure transducer. Physiological saline was infused at room temperature into the bladder at a constant rate of 0.1 ml/min to elicit repeated voiding responses. The volume-evoked micturition reflexes were studied in conscious, restrained rats after allowing at least 2 h for recovery from halothane anesthesia. All experiments were carried out on the same day that the operative procedures were performed.

**Evaluation.** In all experiments, control cystometrograms (CMG) were recorded for 1 to 2 h prior to i.c.v., i.t., and i.v. administration of vehicle or drug solutions. Cumulative dose-response curves were constructed by administering drugs at increasing doses (OXO-M, 0.01–1 µg/rat and atropine, 0.1–30 µg/rat) at 1- to 2-h intervals. In some experiments OXO-M was also tested after atropine pretreatment (15–30 min). Saline voided from the urethral meatus was collected and measured to determine the voided volume (VV). Residual volume (RV) was measured by evacuating the bladder catheter after voiding. RV was measured at least two times during each 30-min period. Bladder capacity (BC) was then calculated as the sum of VV and RV; and voiding efficiency (VE) calculated as BC – RV/B × 100. The intravesical pressure to induce micturition (i.e., pressure threshold, PT) and maximal voiding pressure (MVP) were also measured (Yoshiyama et al., 1999). The pressure immediately after voiding was termed postvoiding intravesical pressure (PVIP, Fig. 1). Bladder compliance was calculated as the amount of infused saline/PT. The presence of nonvoiding contractions, which occurred at a pressure greater than 10 cm of H2O during bladder filling after OXO-M injection, was also noted. These parameters were measured for each CMG.

**Drugs.** Drugs used in this study included halothane (Wyeth-Ayerst Laboratories, Philadelphia, PA), oxotremorine methiodide (a nonselective muscarinic acetylcholine receptor agonist; Research Biochemicals International, Natick, MA), atropine sulfate (Sigma Chemical Co., St. Louis, MO). Oxotremorine-M and atropine sulfate were dissolved in artificial CSF for i.c.v. or i.t. administration and in sterile saline for i.v. administration.

**Statistical Analysis.** Data are expressed as mean ± S.E.M. The changes in the evoked responses after treatment with the drugs were evaluated statistically by means of repeated measures of analysis of variance (ANOVA) followed by Fisher's Protected Least Significant Difference test as a post hoc multiple comparison procedure. The differences among groups were evaluated statistically with the Mann-Whitney U test. A level of p < 0.05 or p < 0.01 was considered statistically significant.

**Results**

**Effects of Intracerebroventricular Administration of Oxotremorine-M.** Under control conditions, mean BC was 0.37 ± 0.04 ml, mean MVP was 38 ± 3 cm of H2O, mean PT was 13 ± 1 cm of H2O, mean PVIP was 11.40 ± 0.6 cm of H2O, and mean VE was 94 ± 2%. Intracerebroventricular, i.e., or i.v. administration of vehicles did not elicit detectable changes in any CMG parameter. The low dose of the drug was ineffective, but higher doses (0.1 and 1 µg) caused a dose-dependent increase in BC (range 100–200%), PT (160–300%), and MVP (45–100%) and a decrease in VE (17–68%) (Figs. 2A and 3). The effect of OXO-M was apparent within 5 min after administration, and the effects were evaluated within the first 30 min. The largest dose (1 µg) of OXO-M also

![Fig. 1. Two types of cystometrograms illustrating various voiding parameters obtained before (A) and after (B) OXO-M injection (1 µg i.c.v.).](image-url)
Effects of Increasing Doses of Oxotremorine-M. The lowest dose of OXO-M (0.01 μg) was ineffective, but a 0.1-μg dose selectively increased BC (85 ± 17%) without altering other parameters. This effect of 0.1 μg of OXO-M persisted for 30 to 60 min (Fig. 4B) but disappeared between 1 and 2 h. OXO-M at a dose of 1 μg increased BC (179 ± 21%), MVP (48 ± 20%), and PT (215 ± 55%) in a dose-dependent fashion (Figs. 2B and 3). The largest dose (1 μg) of OXO-M also caused a significant increase in PVIP from 9.9 ± 0.4 cm of H2O to 16.7 ± 1.2 cm of H2O, and a significant decrease in VE (−11.6 ± 2%), but this effect was significantly smaller than that after i.c.v. administration of OXO-M (Fig. 3). Bladder compliance after injection of 1 μg of OXO-M was significantly smaller (0.05 ± 0.01 ml/cm of H2O) than the preinjection value (0.30 ± 0.06 ml/cm of H2O), whereas smaller doses had no effect. Nonvoiding contractions were noted in only a few animals (one of seven after either 0.1 μg or 1 μg of OXO-M). Following 1 μg of OXO-M, complete recovery of BC and PT occurred 60 to 90 min after administration (Fig. 4B). Pretreatment with atropine at a dose of 1 μg, which had no effect on the CMG, blocked the effects of OXO-M (0.1 and 1 μg) (Figs. 5B and 6B).

Effects of Intrathecal Administration of Oxotremorine-M. Although low doses of OXO-M (0.01 and 0.1 μg) did not significantly change CMG parameters, the highest dose (1 μg) caused small but significant increases in MVP (65 ± 21%), PT (111 ± 29%) (Figs. 1C and 2), and PVIP from 9.4 ± 0.4 cm of H2O to 14.6 ± 1.3 cm of H2O. BC did not change significantly (Fig. 2C). Bladder compliance after injection of 1 μg of OXO-M was significantly smaller (0.04 ± 0.02 ml/cm of H2O) than the preinjection value (0.30 ± 0.04 ml/cm of H2O) whereas smaller doses were ineffective. Nonvoiding contractions were noted in two of five animals after 1 μg of OXO-M. All parameters returned to normal 60 min after i.v. administration.

Effects of Atropine. Small doses of atropine (0.1–1 μg, i.e.v., i.t., and i.v.) did not cause any significant change in CMG parameters. High doses (10–30 μg, i.e.v., i.t., and i.v.) reduced MVP by 31 to 34% and VE by 18 to 25% (Figs. 7 and 8). It is noteworthy that even the highest doses of atropine (i.t. and i.v.) did not cause significant changes in BC. PT and PVIP also were not changed significantly by i.e.v., i.t., or i.v. administration of atropine (data not shown). The effects of atropine (i.v.) on MVP were reversed within 90 min (41.3 ± 3.3 cm of H2O, −7 ± 3% change from pretreatment control) whereas the effects on MVP elicited by i.e.v. or i.t. atropine persisted for 2 h (i.e.v., 27.7 ± 4 cm of H2O, −30 ± 5%; and i.t., 24.7 ± 1.6 cm of H2O, −35 ± 5.6%).

Discussion

The present study demonstrated that activation of muscarinic receptors in the brain and the spinal cord can induce prominent changes in voiding function in the awake rat. Intracerebroventricular or i.t. administration of a muscarinic agonist (OXO-M) had primarily a depressant effect on voiding, although some facilitatory effects were also noted. Atropine in low doses blocked these effects. Higher doses of atropine alone also had a depressant effect. These observations indicate that inhibitory and excitatory muscarinic mecha-
nisms coexist in the central micturition pathways. Muscarinic inhibitory receptors do not appear to be tonically active (i.e. silent receptors) but can be stimulated by administration of muscarinic agonists. On the other hand, the depressant effect of atropine on voiding indicates that muscarinic excitatory mechanisms are tonically active.

Because muscarinic receptors are present in the bladder; one potential concern in the present study was the possibility that drugs administrated into the central nervous system could enter the bloodstream and have effects directly on the bladder. OXO-M was used to reduce this problem because it is a hydrophilic quaternary amine that does not penetrate the BBB as readily as the tertiary amine, oxotremorine, which was used in previous studies (Sillen et al., 1982; O'Donnell, 1990). We believe that only local effects on the brain and the spinal cord were elicited in our experiments because the effects of i.c.v. and i.t. administration of OXO-M were not duplicated by i.v. administration. A previous study reported that doses of OXO-M such as those administered in this study produced effects including hypothermia, tremors, and salivation when administered i.c.v. but not when administered peripherally, indicating a localized effect on the brain (Sanchez and Meier, 1993).

Another potential problem is the spread of drugs within the central nervous system between the spinal and the supraspinal sites. This does not appear to have been a factor in our study, because the onset of the effects of OXO-M was too rapid after both i.c.v. and i.t. administration to be explained by diffusion to other areas. In addition, a moderate dose of OXO-M (0.1 μg, i.c.v.) elicited qualitatively different responses (i.e., increases in BC, PT, and MVP and a decrease in VE) than the response to i.t. administration (a selective increase in BC).

On the other hand, atropine and its quaternary analog both penetrate the BBB (Sanchez and Meier, 1993), so that the potential for systemic effects resulting from muscarinic antagonists after i.c.v. or i.t. administration is greater. Indeed, the time for onset of action and the dose-response curves for effects on MVP and VE were similar for atropine i.c.v., i.t., and i.v. administration. Only BC was selectively increased by i.c.v. administration, indicating a selective action on the brain. This selectivity is also supported by differences in the duration of action; i.e., the effects of i.v. atropine disappeared after 90 min, but those produced by i.c.v. or i.t. administration were still apparent after 2 h. Sanchez and Meier (1993) also reported that the effects of i.c.v. administration of atropine on oxotremorine-induced hypothermia, tremors, or salivation were more than 10 times stronger than those of atropine administered peripherally. Another study found that doses of atropine equivalent to those administered in this study were effective in inhibiting the pressor responses induced by muscarinic agonists when administered i.c.v. but not when administered i.v. (Brezenoff et al., 1988). Thus, it seems reasonable to conclude that atropine admin-
istered by the three routes in our experiments inhibits voiding function by acting at different sites. It is well known that atropine can act on peripheral muscarinic receptors in the bladder muscle to reduce the amplitude of bladder contractions as well as reducing voiding efficiency. Our findings indicate that atropine produces similar effects by acting on the central nervous system. It also increases bladder capacity by blocking muscarinic receptors in the brain.

Our observations regarding the central effects of OXO-M are somewhat different from those reported by other investigators who administered oxotremorine i.v. or i.a. (Sillén et al., 1982; O’Donnell, 1990). A decrease in BC and an increase in MVP was detected after i.v. injection in the rat and in vascular cross-perfusion studies in the dog brain. It seems likely that these studies reflected in large part the peripheral excitatory effects of the muscarinic agonist; whereas our experiments revealed a central inhibitory effect. The most prominent effect of the drug after i.c.v. or i.t. administration was an increase in BC, indicating that the drug increases the central threshold for triggering voluntary voiding. This implies that inhibitory muscarinic receptors can modulate the processing of sensory input from the bladder and suppress the detection of bladder filling. These findings are compatible with those of a previous study (Roppolo et al., 1987), which revealed that injections of carbachol in the region of the locus coeruleus increased BC and suppressed rhythmic bladder contractions in decerebrate cats. Another study (Thor et al., 2000) showed that after blocking peripheral muscarinic receptors, i.v. injection of oxotremorine suppressed reflexes induced by nociceptive stimulation of the bladder. This effect could be elicited by an inhibition of the central processing of afferent input from the bladder and is consistent with other reports of antinociceptive effects of muscarinic agonists administered i.c.v. (Bartolini et al., 1992) or i.t. (Yaksh et al., 1985; Zhou and Gebhart, 1991; Iwamoto and Marion, 1993).

Other effects of OXO-M suggest excitatory actions. For example, the drug stimulated nonvoiding contractions, decreased bladder compliance, and increased MVP. The first two effects could be mediated by stimulation of the parasym pathetic excitatory outflow to the bladder during filling. Thus, OXO-M might elicit two opposing actions on the afferent and efferent limbs of the supraspinal micturition reflex, suppression of the afferent limb to increase the volume threshold for voiding and stimulation of the efferent limb to reduce storage capability of the bladder.

The increase in MVP, coupled with an increase in PVIP and a decrease in VE after OXO-M administration to the brain, is most reasonably explained by a dysfunction of the urethral outlet to increase outlet resistance and reduce the flow of fluid from the bladder. In the rat, rhythmic contractions of the external urethral sphincter are necessary for efficient voiding (Yoshiyama et al., 2000). A previous study found that microinjection of carbachol to locus coeruleus alpha suppressed urethral sphincter activity (Sugaya et al., 1987). Our findings that MVP, PVIP, and nonvoiding contractions were increased and VE reduced by OXO-M suggest that coordinated sphincter activity was reduced by the drug.

The effects of atropine are important for identifying the role of endogenous acetylcholine in the central control of the lower urinary tract. It would be expected that if muscarinic
inhibitory mechanisms are tonically active in controlling voiding function, then atropine would decrease BC and induce bladder hyperactivity. On the contrary, atropine administered i.c.v. increased BC and reduced MVP and VE. This indicates that tonic muscarinic excitatory mechanisms in the brain regulate the afferent and efferent limbs of the micturition reflex pathway, whereas inhibitory mechanisms seem to be inactive. The excitation may occur at sites distant to the pontine micturition center because previous studies (Roppolo et al., 1987) in decerebrate cats did not detect any effect of atropine injected directly into the pons.

The physiological role of muscarinic receptors in the spinal cord is different from in the brain. Administration of atropine to the spinal cord reduced VE and MVP but did not increase BC significantly. This indicates that activation of muscarinic receptors facilitates the efferent rather than the afferent
limb of the micturition reflex pathway in the spinal cord and that acetylcholine may play an active role during voiding but not during urine storage.

In summary, our findings indicate that activation of muscarinic receptors in the central nervous system can inhibit as well as facilitate voiding function in the awake rat. Excitatory muscarinic mechanisms appear to be tonically active to control both the afferent and efferent pathways involved in voiding. On the other hand, the physiological functions of inhibitory muscarinic mechanisms that can be activated by exogenously administered agonists are uncertain because these mechanisms seem to be inactive under normal conditions.

References


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