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ATROPINE AND MICTURITION RESPONSES BY RATS WITH INTACT AND PARTIALLY INNERVATED BLADDER

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1 Micturition responses by a group of 17 rats were recorded during a water diuresis. During a 2 h period, uniform volumes of urine were passed at regular intervals; the mean of the voiding responses by each animal was consistent from one water loading period to another. Residual urine volumes were physiologically insignificant.

2 Atropine treatment did not compromise seriously micturition by water-loaded rats. Treated animals micturated more frequently; the mean volume was 68% of control. The residual urine volume was equal to that of controls.

3 Several weeks after the surgical removal of half the motor innervation of the bladder, there was no significant effect on micturition. Mean voiding volumes were not different from those of controls; residual urine volumes were the same as before denervation.

4 After half the innervation of the bladder had been destroyed, the effect of atropine on micturition was enhanced. Volumes passed were 50% of control; large residual volumes remained when micturition was over. Only in this group could bladder distension be found.

5 It is concluded that functional responses of the rat urinary bladder are not only resistant to atropine but also to the sizeable reduction in the number of neuroeffector units in the bladder itself. The functional reserve of the rat bladder musculature is remarkably high when assessed by its ability to empty adequately.

Introduction

A variety of drugs including anaesthetics and anti-muscarinics can interfere with the reflex pathways subserving micturition. While the effects of these drugs on nerve-induced bladder contractions have been studied extensively *in vivo* and *in vitro*, (Edge, 1855; Ursillo & Clark, 1956; Carpenter, 1963; Huković, Rand & Vanov, 1965; Chesher & Thorp, 1965; Downie & Dean, 1977) their effect on micturition by unrestrained animals have not. The reflex control of the urinary bladder depends on several autonomic pathways. Unlike other autonomic organs, such as the pupil or sweat glands, junctional transmission in the bladder of most species is remarkably resistant to atropine whether the stimuli are applied to both pelvic nerves *in vivo* (Edge, 1955) or by transmural stimulation *in vitro* (Carpenter, 1963; Huković *et al.*, 1965). Furthermore, at low stimulus rates, nerve-induced responses of the bladder are not potentiated by inhibitors of cholinesterase (Ambache & Zar, 1970; Carpenter, 1977; Downie & Dean, 1977). Accordingly, some fraction of the innervation may be non-cholinergic and there is some evidence which suggests a role for 'purinergic' nerves (Burnstock, 1979). This resistance may however be caused by the inaccessibility of the drug to some of the muscarinic receptors (Dale & Gaddum, 1930; Carpenter, 1977; Elmér, 1978). The release of high concentrations of acetylcholine from the nerve terminals of the bladder, in close proximity to its postjunctional receptors, may also contribute to atropine resistance (Huković *et al.*, 1965). In the rat bladder, there is an extensive overlap of the cholinergic innervation (Carpenter & Rubin, 1967); the muscle fibres may respond to transmitter from a large number of nerve terminals during a physiological response. If the density of the cholinergic innervation within the bladder is greater than in other organs, which are not resistant to atropine, the removal of some fraction of its motor nerves might be expected to enhance the blocking action of atropine. To assess the role of the innervation density of the bladder, the action of atropine on micturition was examined before and after half the motor innervation had been destroyed.

Methods

Micturition in conscious rats was recorded after the administration of water equal to 5% of their body weight. The ability of an animal to micturate during the ensuing diuresis was estimated by measuring the volume of urine passed at each micturition and the residual volume of urine following micturition. Both parameters of bladder function were measured in the

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same animals under the following conditions: (1) control, untreated; (2) treated with atropine; (3) after surgical removal of one pelvic nerve and (4) after this denervation and treatment with atropine (2–4 mg/kg i.p.).

Micturition in unrestrained rats

A volume of water, at 35°C, equal to 5% body weight was administered to adult male rats (350–450 g) by stomach tube (a 5 cm length of 3/32 inch o.d. soft copper tubing attached to a syringe). Micturition patterns were obtained after each animal had been placed in specially-constructed metabolism cages which facilitated the maximal recovery of urine. Accumulation of urine in a small plastic beaker beneath the outlet of the metabolism cage activated a force transducer (Figure 1). Food was withheld from the rats in the metabolism cage. This was found to facilitate the recovery of urine. A coarse wire mesh across the funnel prevented contamination of the urine by faeces or hair; the funnel was frequently coated with silicone applied as a fine spray. The output of the transducer was amplified and recorded on an ink-writing oscillograph. An upward deflection of the pen, which resulted from the increased weight of the container, permitted measurements of the volume produced to within 0.2 ml (Figure 2). The recording systems were linear in response throughout the range 0–30 ml. After micturition patterns were obtained for each rat, the recording apparatus was calibrated; 2 ml increments of water from an automatic pipette were delivered to the funnel resulting in proportionate displacements of the pen on the recording paper.

Residual urine, the amount remaining in the bladder after micturition, was measured directly. After micturition, as shown by the pen tracing (Figure 2), the rat was removed from the cage. Any urine in the bladder was expressed by gentle pressure applied between the thumb and forefinger. After collecting the urine on an absorbent paper disc, the volume was determined from the difference in weight before and after collection.

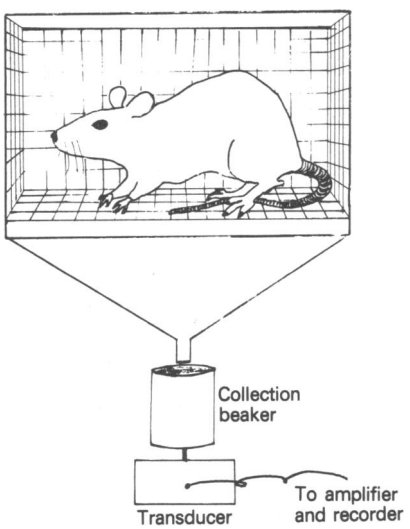

Figure 1 Apparatus for measuring the micturition volume in water-loaded and non water-loaded rats. The front panel which, was raised to remove the animal, is not shown. Throughout the experiments with the non-loaded rats, drinking water was provided by a spout located a short distance from the cage in order to avoid contamination of the collected urine.

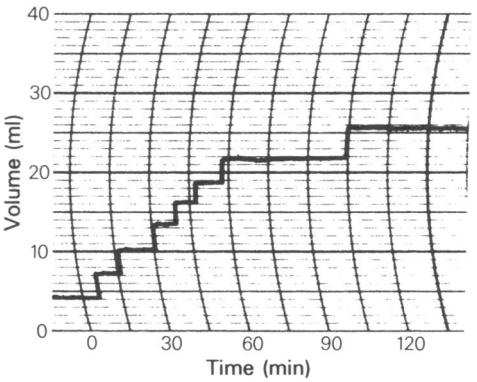

Figure 2 Micturition output by an untreated rat over a 2 h period after the administration by stomach tube of water equal to 5% of the body weight. The volume passed during each micturition response is indicated by an upward deflection of the pen tracing. Each horizontal line corresponds to 1 ml and the vertical lines indicate a period of 15 min.

Unilateral motor denervation of the rat urinary bladder

The left pelvic nerve was sectioned under aseptic conditions in animals anaesthetized with sodium pentobarbitone, 25 mg/kg. Access to the nerve was through a midline incision and the omentum, spermatic cord, and prostate gland were retracted. Filaments of the left pelvic nerve were located microscopically on the lateral surface of the prostate gland close to their origin in the pelvic ganglion. Both nerve and ganglion were cauterized unilaterally. To minimize adhesions, the operative field was irrigated with saline (0.9% w/v NaCl solution). After the abdominal muscles were sutured together with sterile 4–0 chromic gut, the incision was closed by small silver wound clips. Seven days later, after the skin had grown together, the clips were removed and the animal used.

Atropine methyl nitrate, (2 mg and 4 mg/kg i.p.) to diminish antimuscarinic actions within the central nervous system, was given immediately before water loading. Throughout the collection period, the pupils

were periodically examined for mydriasis to confirm the effectiveness of the drug in producing muscarinic blockade. No animals were lost as a result of water loading or from the effects of the surgery; 3 rats died from bacterial infection during the study.

Results

Micturition responses in untreated rats

Untreated rats micturated at regular intervals when they were given water *ad libitum*. Between 15–30 voidings were recorded during a 24 h period; volumes in the range 1.1–2.5 ml were passed and the mean volume voided by 17 animals was 1.4 ± 0.11 ml. After a water load, the mean volume produced over the next several hours amounted to nearly twice that recorded previously in non-loaded rats.

Micturition in water-loaded rats

Control (untreated) rats micturated at regular intervals after a water load was administered (Figure 2). The elimination of urine by the loaded rats appeared constant until most of the load was excreted. The volume passed by each rat was fairly uniform, varying by less than 10%. The point on the chart record (Figure 2) at which micturition first began could be connected with the other points by a straight line. A 450 g rat given 23 ml of water usually eliminated 18 ml or 80% of the load within 1 h. Between 6 and 10 micturition responses usually resulted and the animals passed volumes of urine in the range 2.0–3.7 ml.

The mean micturition volume (MV) is a quantitative indication of the stimulus to initiate micturition. The MV for each rat was obtained from the results of at least 3 water loadings; variation in the micturition volume for each rat was in the range of 5%. In Figure 3, left, the mean for the entire group of 17 rats (2.7 ml ± 0.16) is shown by the height of the open vertical column.

Residual urine and voiding by water-loaded rats

Micturition by control, untreated rats was usually complete and no significant amount of urine could be expressed by manual pressure. In less than 10% of the observations, however, amounts of urine in the range 0.1 ml were recovered. The maximum recorded for this group and in 15 other untreated rats amounted to 0.2 ml. The mean residual urine volume was much less, 0.05 ± 0.01 ml ($n = 32$).

Micturition by water-loaded rats treated with atropine methyl nitrate

Atropine methyl nitrate (2–4 mg/kg) did not cause urinary retention and bladders were never distended after micturition. Thus, rats were able to pass urine as it accumulated during the water loading period. This dose of atropine produced total paralysis of the constrictor pupil throughout the elimination of the water load. Notwithstanding, the ability of the treated rats to micturate was not seriously compromised although the volumes passed were significantly less than control (Figure 3). The MV for the 17 animals treated with atropine was to 1.8 ± 0.1 ml, 68% of the volume measured in the control untreated group (2.7 ml).

Micturition by the atropine-treated rats was complete; in a few cases, between 0.25 and 0.5 ml was found, although the amount was not consistent in the same animal. Higher doses of atropine methyl nitrate (25 mg/kg) did not affect further either the volume of residual urine recovered after micturition or the MV at which micturition was initiated.

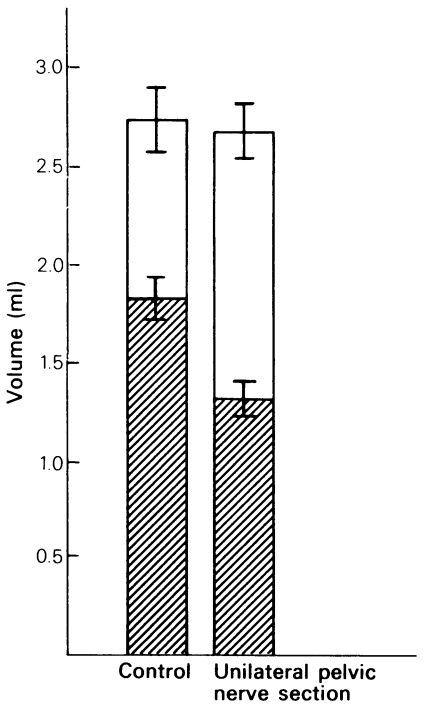

Figure 3 Effects of atropine and unilateral pelvic nerve section on the mean micturition volume of 17 water-loaded rats. Left: the mean of the urine volumes collected after each period of micturition by 17 water-loaded rats before (open) and after 2 mg/kg atropine methyl nitrate i.p. (hatched). Right: the mean of the urine volumes after each period of micturition by the same water-loaded animals following surgical section of the left pelvic nerve 7 days previously (open) and after treatment with 2 mg/kg atropine methyl nitrate i.p. (hatched). The difference between both groups of rats treated with atropine was significant ($P < 0.005$), vertical lines show s.e. mean.

After atropine, the contractile force of the bladder during a voiding response appears to be adequate to expel urine effectively. Nevertheless, the mean volume stimulus which elicted the responses was significantly less than in the untreated rats. However, the difference between control and atropine-treated rats could not be accounted for by inadequate emptying. The residual urine was never sufficient to account for the smaller voiding volume.

Micturition in rats after partial denervation of the bladder

After both pelvic nerves had been sectioned, the bladder became distended and micturition ceased. Urine is passively expelled through the external sphincter by the high intravesical pressure (Carpenter & Rand, 1965). In contrast, there was no effect on micturition if only one pelvic nerve was sectioned. Micturition by the same animals used in the control and atropine experiments was examined one week after section of the left pelvic nerve. The MV (Figure 3, right column) obtained 7 days after section was not different from control. In this group, the mean volume from the 17 rats was 2.6 ± 0.13 ml as shown to the right in Figure 3 (open column). The immediate effects of unilateral pelvic nerve section were not observed because these rats could not be safely water loaded. Moreover, micturition was judged to be complete since the amount of residual urine recovered did not differ from controls.

Micturition in rats after partial denervation and atropine

After one pelvic nerve had been sectioned, atropine methyl nitrate produced a significant limitation in bladder function. The MV of this group was only 1.3 ± 0.06 ml, half that of the untreated control group (2.7 ml) and of the group that had received unilateral pelvic nerve section but no atropine (2.6 ml). Atropine reduced the micturition volume by more in the denervated than in the control group. Micturition by the animals of the denervated group after atropine was always incomplete; volumes of urine in the range 0.5 to 3.5 ml were recovered. Although the volume of residual urine recovered was found to vary, the bladder was almost always distended after micturition was over.

The MV obtained for each of the four groups (Figure 3) suggests that: (1) the control group was significantly different after atropine was administered; (2) the denervated group was significantly different after atropine was administered; and (3) there was a significant difference between the control and denervated groups after treatment with atropine. A two way analysis of variance was performed with repeated measures of both factors, atropine and nerve section. There was a significant atropine effect ($P < 0.005$), and a significant potentiation of atropine by nerve section. That is, the administration of atropine to animals with unilateral pelvic nerve section produced a greater effect than the sum of each effect alone ($P < 0.005$).

Micturition frequency in untreated and unilaterally-denervated rats after atropine

The elimination of the water load by control, atropine-treated and operated rats was linear with time (Figure 2). Micturition by all 4 groups occurred at regular intervals in proportion to the micturition volume. Thus, fewer responses were elicited by an untreated rat in which MV was large (3.5 ml) than by a rat in which MV was small (< 2 ml). Furthermore, micturition occurred more often in the atropine-treated (MV = 1.8 ml) than the untreated rats (MV = 2.7 ml). This relation between micturition frequency and volume was especially apparent after unilaterally denervated rats were treated with atropine. During the elimination of water load, twice as many micturition responses occurred in the denervated animals after atropine than occurred in these animals when given no drug.

Discussion

Micturition occurs in all mammals very soon after birth yet little is known of the factors which regulate this response. In water-loaded rats the mean voiding volume was roughly twice that of animals given water *ad libitum*. In the former group, urine was eliminated at around 15 ml/h while in the same animals, not water-loaded, at less than 2 ml/h. Urine enters the bladder almost eight times as rapidly during the diuresis but the volume stimulus required to initiate voiding is apparently increased almost two fold. It would indeed appear that stretching of the bladder wall alone is not the sole determinant of a voiding response.

The use of conscious animals for the study of drug actions on the bladder is probably more physiological than the use of isolated preparations. Nerve-induced responses elicited *in vitro* at optimal stimulus rates are usually maximal since nearly all the motor units of the bladder are made to respond to the stimulating current. On the other hand, a maximal response of the bladder may not be initiated during reflex micturition. Thus one might predict a more striking action of atropine on *in vivo* responses. Surprisingly, the MV was diminished by only 32% after atropine with no significant increase in residual urine. The measurement of residual urine is only an estimate of voiding efficiency. Moreover, the volume remaining at the end of voiding may not be completely recovered. Despite every effort

to express as much of the urine from the bladder as possible, some may remain, especially if the organ had been greatly distended. Nevertheless, residual urine was not significantly different from control after atropine or unilateral pelvic nerve section. However, there was a much greater volume of residual urine after the unilaterally denervated animals were treated with atropine. This finding was the most significant functional impairment found.

In other species, the usual elevation in bladder pressure during filling may be diminished by spinal reflexes mediated by sympathetic inhibitory fibres in the hypogastric nerve. One reflex is believed to act directly upon the bladder musculature (Edvardsen, 1968) while another may inhibit transmission through the pelvic ganglia (De Groat & Saum, 1976). Although nerve-induced contractions of the rat bladder *in vitro* are antagonized by noradrenaline or isoprenaline (Carpenter, 1970), it is doubtful whether the sympathetic innervation suppresses bladder contractility associated with micturition in the rat; adrenergic fibres do not seem to influence intravesical pressure at physiological stimulus rates (Elmér, 1978). Also in other species, the urinary bladder may contract during stimulation of the hypogastric nerves (Sigg & Sigg, 1964; Shaffer, Stephenson & Thomas, 1979). Bladder contractions mediated by α-adrenoceptors would appear to be restricted to the trigone at the base of the organ (Raezer, Wein, Jacobowitz & Corriere, 1973) and produce small elevations in pressure. Moreover, contractions of the rat bladder elicited by the hypogastric nerves appear to be mediated by cholinergic fibres (Elmér, 1978). The effect of atropine on voiding contrasted sharply with that of many central depressants such as morphine. At a dose of 5 mg/kg, morphine sulphate increases micturition volume some 2–4 fold in most of the rats and completely abolished micturition in the others (Carpenter, unpublished observations). Morphine clearly increases the threshold for micturition while it would appear that atropine lowers it. There was little to suggest from these experiments that atropine interfered with the micturition reflex in a manner comparable to morphine. Indeed a large micturition volume and/or urinary retention suggest an action by morphine on the central pathways essential for micturition.

Since atropine methyl nitrate does not easily cross the blood-brain barrier, its action must be confined largely to muscarinic receptors in the periphery. Nerve-induced contractions of the rat bladder produced *in vitro* at optimal stimulus rates are not only reduced some 30–40% after atropine but are maintained for shorter periods of time (Carpenter, 1977). The micturition patterns of atropine-treated animals were similarly different from controls; the mean volumes passed by the former were 32% less than the latter. In both cases, atropine only partially blocks neuroeffector transmission resulting in lower pressures during responses of reduced duration. Accordingly, rats treated with atropine, passed less urine during micturition but no significant amount of urine remained afterwards. A smaller micturition volume is thus in agreement with the action of atropine on nerve-induced responses *in vitro* but a significant increase in residual urine would also have been expected.

Section of one pelvic nerve in the rat uniformly reduces the density of the motor innervation throughout the bladder musculature. Each nerve is distributed evenly to both sides of the organ and the innervation by one nerve overlaps the innervation of the other by as much as 30% (Carpenter & Rubin, 1967). Although removal of half the motor and sensory innervation did not noticeably interefere with voiding, the responsiveness of the bladder itself to stimulation of the remaining motor nerves *in vitro* is nevertheless diminished; only half as much contractile force is produced during transmural stimulation (Carpenter & Rubin, 1967). Thus, the pressure developed during voiding may be less but it is apparently adequate to empty the bladder completely. Indeed, the same may be said after a partial blockade of transmission in the bladder was produced by atropine. These findings suggest that voiding responses in conscious rats have a large margin of safety.

Water-loaded rats would appear to be useful as models for the study of drug action on micturition. The action of atropine on bladder function of conscious rats is consistent with its action on the bladder *in vitro*. Although the rats appeared to void normally with only half their bladder innervation intact, the functional capacity of the bladder was compromised nevertheless; voiding volumes were even further dininished to only 50% of control by atropine and residual urine volumes increased markedly. The appearance of both factors following atropine suggest an unmasking of a functionally significant deficit in the partially denervated bladders. Conversely, the diminished functional capacity of the bladder following partial muscarinic blockade, became proportionately greater after its motor innervation was reduced by half.

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