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RESPONSES OF THE RAT URINARY BLADDER *IN SITU* TO DRUGS AND TO NERVE STIMULATION

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The autonomic innervation of the urinary bladder has been studied extensively. Langley & Anderson (1895) first showed that stimulation of the pelvic nerves produced contraction of the bladder. Elliot (1907) reported considerable species differences in the innervation of the bladder. The responses of the urinary bladder of several mammalian species (dog, cat, rabbit and ring-tail possum) to drugs and to nerve stimulation have been investigated by Edmunds & Roth (1920), Henderson & Roepke (1935), Edge (1955), Ursillo & Clark (1956) and Burnstock & Campbell (1963).

The present paper describes the actions of acetylcholine, nicotine, dimethylphenylpiperazinium and 5-hydroxytryptamine on the rat urinary bladder *in situ*. Responses of the bladder were elicited by stimulation of its motor nerves and compared with the responses produced by drugs. The results indicate that the autonomic innervation of the urinary bladder of the rat has characteristics of its own.

METHODS

Rats of either sex weighing 180 to 250 g were used. They were anaesthetized by pouring a few drops of halothane on cotton wool in a Perspex box (dimensions $20 \times 15 \times 15$ cm). Complete anaesthesia was obtained within 5 min. The rats were not atropinized because halothane, unlike ether, did not cause mucous secretion. A cannula was inserted into the trachea, the rats were pithed as described by Shipley & Tilden (1947) and immediately put on artificial ventilation. A polyethylene cannula was inserted into a femoral vein for injecting drugs. The blood pressure was recorded from a carotid artery with either a Condon manometer or a pressure transducer (Devices).

The lower abdomen was opened along the midline, and the bladder exposed and detached from the prostate gland by blunt dissection. A thread attached to the vertex was fixed to a lever or to a strain-gauge for recording isotonic contractions, at a tension of 1.5 to 2 g, on a kymograph or a pen-recorder. Cotton-wool swabs soaked with warm saline were laid around the bladder to keep it warm and moist.

The vesical nerves were stimulated by shielded platinum electrodes put underneath a ureter a few millimetres from the bladder. Rectangular pulses of 1 to 2 msec duration, and 5 to 10 V strength, generated from a Palmer electronic stimulator, were applied at a frequency of 10 or 20 shocks/sec, for 10 sec every 2 min.

The hypogastric nerve was stimulated at about 1 cm from the bladder with pulses of 2 msec duration, 5 to 10 V strength and a frequency of 10 to 50 shocks/sec. In several experiments the right cervical vagus was stimulated. The stimuli applied were of the same duration, strength and frequency as those for the vesical nerves.

The nerves were not cut, but stimulation was in effect only of centrifugal fibres, because the central nervous system in these animals had been destroyed by pithing.

The drugs used were acetylcholine chloride, adrenaline, anabasine tartrate, atropine sulphate, 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride (McN-A-343), dimethylphenylpiperazinium iodide, physostigmine salicylate, guanethidine sulphate, hemicholinium dibromide, hexamethonium bromide, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, methysergide (UML 491, Sandoz), morphine sulphate, neostigmine bromide, nicotine hydrogen tartrate, noradrenaline bitartrate, pempidine tartrate and procaine hydrochloride. Halothane was used as Fluothane (I.C.I.). The amounts of catechol amines, anabasine and nicotine are given in terms of bases. The doses of all other drugs are given in terms of their corresponding salts. The drugs for injections were dissolved in 0.9% saline. The volume of a single injection did not exceed 0.3 ml.

RESULTS

Responses to drugs

The responses of the rat bladder to intravenous injections of drugs are shown in Table 1. The drugs can be divided into three groups according to their effect: those producing contraction, those producing spasm, and those which were either without effect or produced

TABLE 1

EFFECTS OF DRUGS ON THE RAT URINARY BLADDER *IN SITU*

* Determined by comparing doses (in terms of bases, see Methods) producing the same effect. McN-A-343=4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride. Injections were intravenous

Drug	Dose (μg)	Effect	Relative potency*
Acetylcholine	0·0025– 0·1	Contraction	100
5-Hydroxytryptamine	0·25 – 4	Contraction	12
Dimethylphenyl-piperazinium	2·5 – 25	Contraction	0·4
Nicotine	2·5 – 50	Contraction	0·2
Anabasine	10 –250	Contraction	0·04
Physostigmine	10 – 50	Spasm	—
Neostigmine	10 – 20	Spasm	—
Adrenaline	0·001 – 0·03	None or small relaxation	—
Noradrenaline	0·001 – 0·03	None or small relaxation	—
Histamine	10 – 30	None or small contraction	—
McN-A-343	10	None	—

very small effect (denoted as " small contraction " or " small relaxation "). Only the drugs of the first group produced regular responses, amenable to quantitative analysis. Their relative potency is given in the fourth column.

The contractions of the bladder following the injections of acetylcholine, 5-hydroxytryptamine, nicotine or dimethylphenylpiperazinium were often strong enough to cause voiding of urine when the bladder contained any. After larger doses of 5-hydroxytryptamine, nicotine or dimethylphenylpiperazinium, retraction of the scrotum in the male was observed. The site of action of the drugs in causing this reaction was not investigated.

Acetylcholine. This caused rapid contractions of the bladder (Fig. 1) which coincided with falls in blood pressure. The threshold dose for the contractions of the bladder varied from 2.5 to 100 ng. The height of the contractions was proportional to the dose of acetylcholine. Atropine (5 to 10 mg/kg) abolished the responses of the bladder and the blood pressure to small doses of acetylcholine (Fig. 1), but larger doses (0.5 to 1 mg) again contracted the bladder and caused a rise in blood pressure. Physostigmine (0.05 to 0.25 mg/kg) potentiated the bladder contractions produced by acetylcholine.

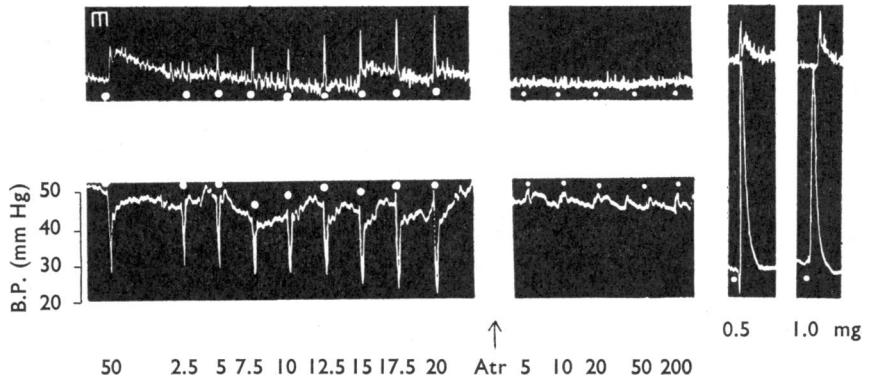

Fig. 1. Responses of the bladder (above) and blood pressure (B.P., below) to intravenous injections of acetylcholine (doses in ng). Atropine (Atr), 2 mg, was injected at the arrow and abolished the responses to small doses of acetylcholine. The two right-hand panels show the effects of large doses of acetylcholine. Time marks in minutes.

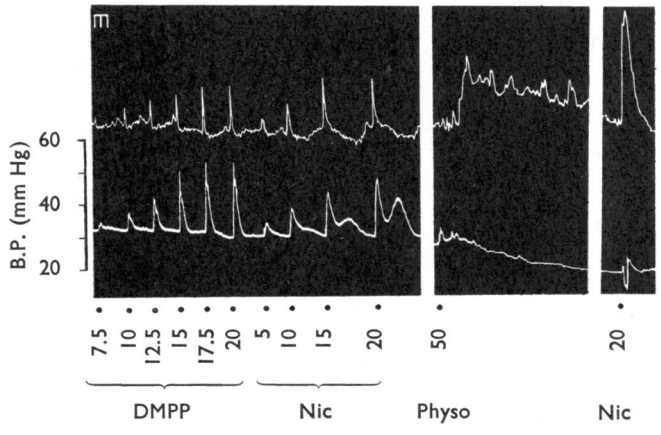

Fig. 2. Responses of the bladder (upper trace) and blood pressure (B.P., lower trace) to dimethylphenylpiperazinium (DMPP), nicotine (Nic) and physostigmine (Physo). The contraction of the bladder produced by nicotine was enhanced after physostigmine. Time marks in minutes. Doses in μg.

Anticholinesterases. Doses of 10 to 50 μg of physostigmine (Fig. 2) or 10 to 20 μg neostigmine produced sustained spasms of the bladder. There was also a slight increase of the blood pressure followed by a prolonged decline. These doses of physostigmine or neostigmine often caused fasciculations of the skeletal muscles and deterioration of the preparation. Atropine or hyoscine (5 mg/kg) counteracted the spasm of the bladder.

Nicotine and dimethylphenylpiperazinium. These caused contractions of the bladder and rises in blood pressure (Figs. 2 and 5). The dose/response curves for contractions of the bladder are shown in Fig. 3 and differ in shape from the curves for acetylcholine and 5-hydroxytryptamine. Repeated injections of the same dose of either drug did not always give uniform responses. The first few doses in an experiment produced gradually increasing responses. If the injections were given at short intervals, and especially after larger doses

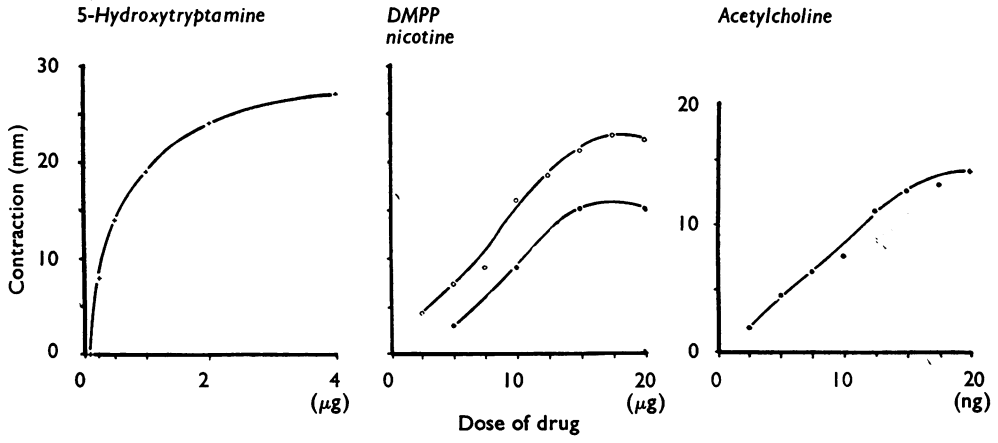

Fig. 3. Dose/response curves for drugs acting on the bladder. The ordinates show the contractions of the bladder in millimetres on the kymograph. The abscissae indicate the amounts of drugs injected: in micrograms (for nicotine, dimethylphenylpiperazinium (DMPP) and 5-hydroxytryptamine) or nanograms (for acetylcholine).

of nicotine, the responses tended to decrease. Hexamethonium (10 mg/kg), pempidine (10 mg/kg) or a large dose of nicotine (5 mg/kg) blocked the responses of bladder and blood pressure to nicotine (Fig. 5) and to dimethylphenylpiperazinium. Atropine or hyoscine, in doses sufficient to abolish the effects of acetylcholine, reduced but did not abolish the contractions of the bladder caused by nicotine or dimethylphenylpiperazinium. This partial blockade by hyoscine (or atropine) was often short-lasting, as shown in Fig. 5. Physostigmine in doses of 0.05 to 0.25 mg/kg (Fig. 2) or neostigmine (0.05 to 0.1 mg/kg) potentiated and prolonged the contractions of the bladder elicited by nicotine or dimethylphenylpiperazinium. Adrenergic neurone blocking agents (guanethidine or bretylium, 10 mg/kg) were without effect.

Anabasine. This is a nicotine-like alkaloid occurring in tobacco and tobacco smoke. It had similar actions on the bladder and the blood pressure to those of nicotine, but was less potent (Table 1).

5-Hydroxytryptamine. The actions of 5-hydroxytryptamine on the bladder and blood pressure are shown in Fig. 4. Successively increasing doses of the amine produced graded responses consisting of contractions of the bladder and rises in blood pressure. The dose/response curve for 5-hydroxytryptamine differs in form from that of acetylcholine (Fig. 3). Hexamethonium (10 mg/kg) did not affect the responses to 5-hydroxytryptamine. Atropine in a dose of 5 to 10 mg/kg reduced the responses to 5-hydroxytryptamine by one-third or one-half of their initial height. Morphine partially antagonized the action of 5-hydroxytryptamine in contracting the bladder (Fig. 4). The pressor response to 5-hydroxytryptamine appeared to be slightly enhanced by morphine (Fig. 4). Methysergide together with morphine abolished the effects of 5-hydroxytryptamine on the bladder and blood pressure. In another experiment methysergide was injected before morphine; it reduced the response of the bladder and blood pressure to 5-hydroxytryptamine. Then morphine completely abolished the responses.

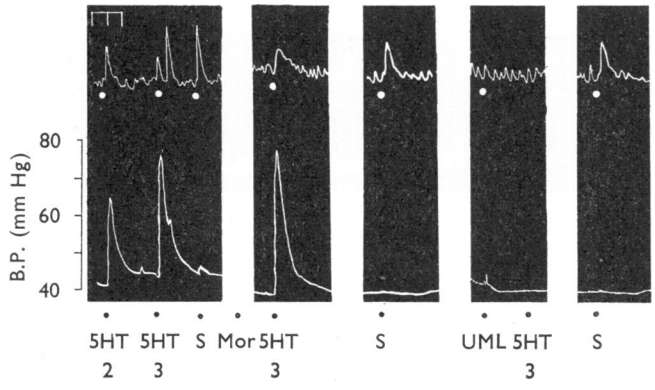

Fig. 4. Effects of morphine (Mor, 2 mg) and methysergide (UML, 100 μg) on the responses of the bladder (upper trace) and blood pressure (B.P., lower trace) to 5-hydroxytryptamine (5HT) in the doses indicated in micrograms and to stimulation of the vesical nerves (at S) with 2-msec, 5-V pulses at 20 shocks/sec for 10 sec. Time marks in minutes.

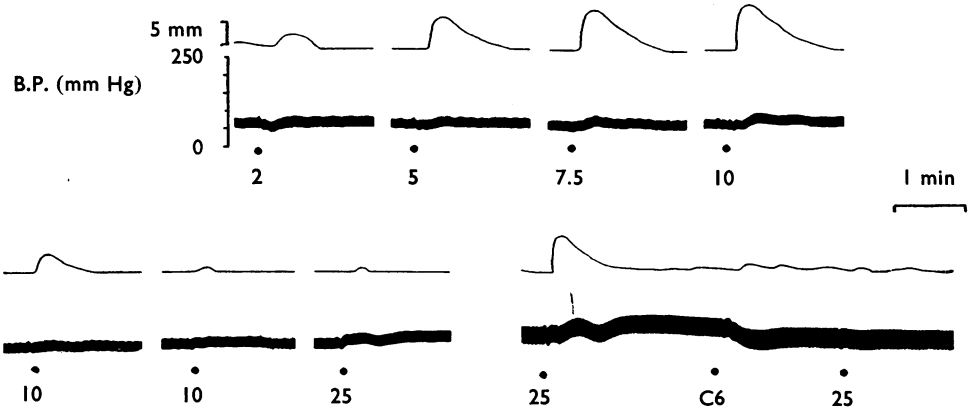

Fig. 5. Responses of the bladder (upper trace) recorded with an isotonic displacement transducer and blood pressure (B.P., lower trace) recorded with a pressure transducer. Nicotine was injected intravenously in the doses (μg) indicated by the numerals. Upper panel: initial observations. Lower left-hand panel: after hyoscine; first record after 250 μg hyoscine, second and third records after an additional dose of 1 mg of hyoscine. Lower right-hand panel: the response to 25 μg nicotine 15 min after the previous dose, which is shown on the previous panel, has increased owing to the effect of hyoscine wearing off. Then hexamethonium (C6, 10 mg/kg) abolished it.

Catechol amines. The pithed rat is extremely sensitive to the pressor action of noradrenaline and adrenaline. Doses of up to 30 ng were usually without effect on the bladder, but in a few experiments they caused a small relaxation.

McN-A-343. This drug is a ganglion stimulant reported to act selectively on sympathetic ganglion cells (Roszkowski, 1961). In a dose of 10 μg it produced a marked increase in the blood pressure, but was without effect on the bladder.

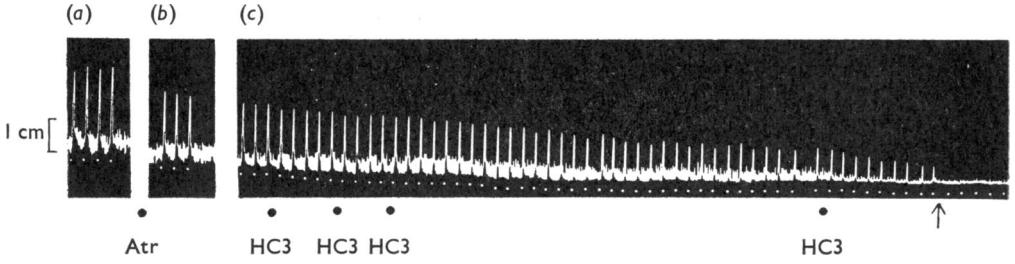

Fig. 6. Contractions of the bladder produced by stimulation of the vesical nerves (at white dots) with 2-msec, 5-V pulses at 20 shocks/sec for 10 sec every 2 min. Initial observations are shown in (*a*). Atropine (Atr, 10 mg/kg) injected between (*a*) and (*b*) partly reduced the contractions. In (*c*), hemicholinium (HC3) was given in four injections each of 5 mg/kg; there was a gradual reduction in the contractions and, after stimulation for 1 min at the arrow, the contractions failed completely.

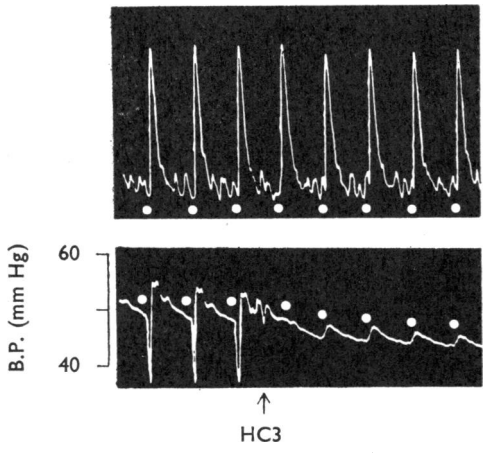

Fig. 7. Effect of hemicholinium (HC3, 10 mg/kg) on the contractions of the bladder produced by stimulation of the vesical nerves (upper trace) and the falls in the blood pressure (B.P., lower trace) produced by stimulation of the right cervical vagus with 2-msec, 5-V pulses at 50 shocks/sec for 10 sec every 2 min, at the white dots.

Responses to nerve stimulation

Vesical nerves. Electrical stimulation of the vesical nerves adjoining the ureter produced prompt contractions of the bladder (Fig. 6). These contractions had the same characteristics as the contractions of the isolated bladder elicited by stimulation of the vesical nerves (Huković, Rand & Vanov, 1964). They were unaffected by ganglion-blocking drugs, by methysergide (Fig. 4) or by guanethidine, but were potentiated by physostigmine. Atropine (Fig. 6) or hyoscine only partly blocked these responses. Hemicholinium (20 mg/kg) caused the responses to vesical nerve stimulation to fail gradually, until finally, after about 2 hr of repeated stimulation, a complete block was obtained (Fig. 6). Fig. 6 also shows that hemicholinium was effective against the atropine-resistant responses. The vesical contractions caused by nicotine or dimethylphenylpiperazinium were absent in the animals treated with hemicholinium (20 to 30 mg/kg) at the time when the responses

to nerve stimulation were also blocked. The responses to the stimulation of the vesical nerves were greatly reduced or prevented after procaine (2 mg/kg intravenously or a few drops of 1% solution applied topically to the bladder), or after ligating the ureter between the electrodes and the bladder.

Hypogastric nerve. Stimulation of one or both of the hypogastric nerve trunks produced little or no effect on the bladder. The efficacy of the stimulation was shown by observations on other pelvic organs innervated by the hypogastric nerves.

Vagus nerve. Stimulation of the right cervical vagus was without effect on the bladder. In one experiment (Fig. 7) both the right vagus and the right vesical nerve were stimulated. Stimulation of the vagus resulted in falls of blood pressure and stimulation of the vesical nerves produced contractions of the bladder. Then, hemicholinium (10 mg/kg) was injected. The vagal responses were blocked immediately, but the vesical contractions remained unaltered. In order to block the vesical contractions elicited by nerve stimulation, repeated stimulation of the nerves was necessary (for 2 hr or more) and a higher dose of hemicholinium was required (compare Figs. 6 and 7).

DISCUSSION

Most studies on mammalian urinary bladder have been made using dogs or cats with the bladder *in situ* (Langley & Anderson, 1895; Elliot, 1907; Edmunds & Roth, 1920; Henderson & Roepke, 1935; Edge, 1955; Gyermek, 1961). Responses to drugs and to nerve stimulation have usually been recorded by measuring changes in the intravesical pressure. In the present experiments, using the rat, the responses of the urinary bladder were recorded as contractions in the axis from the urethra to the vertex. The use of pithed rats excluded central and reflex influences, so that the effects of drugs were confined to peripheral mechanisms. Simultaneous recording of the blood pressure and the movements of the bladder gave information about the actions of nicotinic drugs at different sites. Thus, the rise in blood pressure and the contraction of the bladder produced by dimethylphenylpiperazinium can be used as criteria of excitation of sympathetic and parasympathetic ganglia respectively (Chen, Portman & Wickel, 1954; Garret, 1963).

Before pithing, the rats were anaesthetized with halothane. In contrast to ether, halothane induces rapid anaesthesia without mucous secretion (Raventos, 1956). The usual pretreatment with atropine was therefore avoided and the actions of drugs with muscarine-like actions could be investigated.

In species other than the rat, contractions of the bladder have been observed on stimulation of the pelvic nerve, hypogastric nerve (see Gruber, 1933; Langley & Anderson, 1895; Elliot, 1907; Edge, 1955; Garret, 1963) and even the vagus nerves (Oehl, 1865, and Palumordwinov, 1916; cited in Gruber, 1933). The absence of responses of the bladder to stimulation of the vagus in the rat shows that in this species the vagus does not contribute motor fibres to the bladder. The possibility of vagal fibres innervating the bladder of other species was discounted by Gruber (1933). Elliot (1905; cited in Gruber, 1933) observed that stimulation of the hypogastric nerve in the rat was without effect on the bladder. The present experiments confirm Elliot's observation, from which it appears that the hypogastric nerves in the rat, in contrast to other species, do not carry excitatory fibres to the urinary bladder. On the other hand, stimulation of the vesical branches of the pelvic

nerves provoked marked motor responses of the bladder. These responses were unaffected by ganglion-blocking drugs, methysergide or guanethidine, which suggests that the peri-uretal vesical nerves in the rat contain postganglionic cholinergic fibres. The contractions of the bladder elicited by stimulation of the vesical nerves were resistant to the blocking action of atropine or hyoscine. Such an atropine resistance is known to occur with other cholinergic nerves and by itself does not exclude the cholinergic nature of the responses (Ambache, 1955). An explanation of the atropine-resistance suggested by Huković *et al.* (1964) was that the acetylcholine released by nerve impulses may reach high concentration locally, sufficient to surmount the blockade of the receptors by atropine. As shown in the present experiments, the blockade of the muscarinic receptors in the bladder by atropine is not absolute and large doses of acetylcholine overcame it. The atropine-resistant responses to nerve stimulation were susceptible to the blocking action of hemicholinium.

The contractions of the bladder of the dog on administration of nicotine (Larson, Haag & Silvette, 1961; Gyermek, 1961) or dimethylphenylpiperazinium (Chen *et al.*, 1954; Gyermek, 1961; Garret, 1963) were believed to be indirect via excitation of autonomic ganglia from which motor fibres to the bladder arise. The present experiments in the rat also point to a ganglionic site of action both for nicotine and dimethylphenylpiperazinium. The actions of both drugs were blocked by ganglion-blocking drugs, or by a large dose of nicotine itself. The facilitation observed with small doses also suggests a ganglionic action. A direct action on the smooth muscle of the bladder is unlikely, since both nicotine and dimethylphenylpiperazinium were practically inactive on the rat isolated bladder (Huković *et al.*, 1964). The question then arises on which ganglia nicotine and dimethylphenyl-piperazinium act to cause contraction of the bladder. Possible sites of action are the cells of the sympathetic inferior mesenteric ganglion and the parasympathetic ganglion cells of the pelvic plexus. It is unlikely that sympathetic ganglion cells are involved, since there were no responses to hypogastric nerve stimulation, nor to injections of adrenaline or noradrenaline. The ganglion-stimulant drug, McN-A-343, reported to excite only sympathetic ganglia (Roszkowski, 1961), was without effect on the bladder in the dose which caused a marked pressor response. Guanethidine or bretylium blocked only the effects of nicotine and dimethylphenylpiperazinium on the blood pressure, but did not influence their effects on the bladder. All these observations suggest that the contractions of the bladder caused by nicotine or dimethylphenylpiperazinium are not mediated by sympathetic, adrenergic nerves. There is evidence that the responses of the bladder to nicotine and dimethylphenylpiperazinium are due to stimulation of ganglion cells in the pelvic plexus which discharge motor impulses to the bladder via the vesical nerves. The contractions elicited by vesical nerve stimulation and by nicotine or dimethylphenyl-piperazinium thus have a common final path. They were potentiated by anticholinesterases, were reduced by atropine or hyoscine and were blocked by hemicholinium.

The powerful contractile responses of the bladder in the pithed rats to 5-hydroxy-tryptamine are in sharp contrast to its weak action on the isolated bladder (Huković *et al.*, 1964). This discrepancy in the activity of 5-hydroxytryptamine *in situ* and *in vitro* suggests that the amine in the pithed rat acts on autonomic ganglia in causing contractions of the bladder. Trendelenburg (1957) has demonstrated that 5-hydroxytryptamine excites ganglia, although it does not act on nicotinic receptors. This may explain why the action of 5-hydroxytryptamine was unaffected by hexamethonium. Trendelenburg (1957) and

Gyermek (1962) have shown that morphine antagonizes the ganglionic action of 5-hydroxytryptamine. In the present experiments morphine reduced the contractions of the bladder due to 5-hydroxytryptamine. After morphine, 5-hydroxytryptamine produced slow contractions of the bladder, similar to those seen on the isolated organ (Huković *et al.*, 1964). Since these responses after morphine were readily blocked by methysergide they are presumably a result of a direct musculotropic action of 5-hydroxytryptamine.

SUMMARY

1. The autonomic innervation of the urinary bladder *in situ* was studied on pithed rats by analysing its responses to nerve stimulation and to intravenous drugs, with simultaneous recording of blood pressure.

2. Acetylcholine provoked contractions of the bladder, which were blocked by atropine and potentiated by physostigmine.

3. The contractions of the bladder produced by nicotine or dimethylphenylpiperazinium were blocked by hexamethonium or pempidine, potentiated by physostigmine and partly blocked by atropine or hyoscine. These results suggest that nicotine and dimethylphenylpiperazinium act on parasympathetic ganglia from which motor fibres to the bladder arise.

4. 5-Hydroxytryptamine produced contractions of the bladder, which were reduced by morphine alone, and abolished by a combined treatment of morphine and methysergide. The results suggest that 5-hydroxytryptamine has a dual action in causing contraction of the bladder: stimulation of the ganglia, although not via nicotinic receptors, and a direct action on the smooth muscle of the bladder.

5. Electrical stimulation of the vesical nerves produced contractions of the bladder, which were enhanced by physostigmine, reduced by atropine or hyoscine, and blocked by hemicholinium. Stimulation of the cervical vagus or of the hypogastric nerves was without effect on the bladder.

6. It is concluded that, in contrast to other species, the urinary bladder of the rat receives a single motor cholinergic innervation by way of the vesical branches of the pelvic nerves.

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