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OBSERVATIONS ON AN ISOLATED, INNERVATED PREPARATION OF RAT URINARY BLADDER

BY

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Preparations of isolated tissues together with their extrinsic nerves are useful for obtaining information about the autonomic innervation and the mode of action of various drugs. Ursillo & Clark (1956) described a preparation of rabbit bladder consisting of a strip of the detrussor muscle together with its periarterial nerves, and Burnstock & Campbell (1963) studied the whole isolated bladder of the marsupial possum together with the periarterial nerves accompanying the blood vessels to the urethra and to the fundus. The isolated innervated preparation of the rat urinary bladder has been used previously for the study of drug action (Bobarević, Huković & Deželić, 1962; Huković & Bubić, 1963) and it was demonstrated to the British Pharmacological Society by Huković, Rand & Vanov in 1964. This paper gives a further description of the preparation and its responses to drugs with reference to the autonomic innervation of the bladder.

METHODS

Male or female rats were killed by a blow on the head and bled. The lower abdomen was opened along the midline to expose the bladder. A thread was tied around the urethra for attaching the bladder in an organ-bath. One ureter was dissected for about 1 cm from the bladder, and a thread was attached for passing the ureter through a bipolar electrode of the type described by Burn & Rand (1960). Nerves to the bladder run close to the ureter, but in the rat they are not identifiable by the naked eye. A thread was sewn through the vertex of the bladder for attaching the preparation to a writing lever. The bladder was then dissected free and set up in a 25-ml. organ-bath containing McEwen's (1956) solution gassed with 95% oxygen and 5% carbon dioxide. The temperature was 33 to 35° C. The writing-lever exerted a tension of 1.5 g on the tissue. The magnification was five- to seven-times. Electrical stimulation was given from electronic stimulators generating rectangular pulses: details of the stimulus parameters are in Results.

Extraction and estimation of acetylcholine. The bladder was excised rapidly, weighed and extracted for acetylcholine by the method of Beani & Bianchi (1963). In two experiments there was complete recovery of a known amount of acetylcholine added to half of a bladder immediately before extraction; the amount of acetylcholine added (100 ng) was approximately equal to the amount found in bladders.

The extracts were assayed against acetylcholine in causing contraction of the guinea-pig ileum and a fall of blood pressure in the pithed rat. The ileum was sensitized with physostigmine (0.02 μg/ml.) added to the reservoir of Tyrode solution. As recommended by Beani & Bianchi (1963), the specificity of the ileal responses was improved by adding diphenhydramine hydrochloride (0.02 μg/ml.) and morphine (0.05 μg/ml.) to the Tyrode solution.

Drugs. The amounts of nicotine and catechol amines referred to in Results are expressed in terms of the bases. The amounts of other drugs refer to their corresponding salts: acetylcholine chloride, atropine sulphate, bretylium tosylate, choline chloride, dimethylphenylpiperazinium iodide, physostigmine salicylate,

guanethidine sulphate, hemicholinium dibromide, hexamethonium bromide, 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, morphine sulphate and procaine hydrochloride. Synthetic bradykinin (BRS 640, Sandoz) and methysergide (UML-491, Sandoz) were used from ampoules.

Drugs were dissolved either in 0.9% saline or in distilled water. Dilutions for application to the organ-bath were made in McEwen's solution.

RESULTS

Effects of drugs

Acetylcholine. This caused an immediate contraction of the bladder (Figs. 1, 2, 3 and 6), the response taking 15 to 20 sec to reach its peak. After replacing fresh McEwen's solution in the bath the bladder relaxed to its initial tone. Inspection of the bladder showed that all parts contracted, but the most prominent component was in the direction of attachment (that is parallel to the axis from urethra to vertex). The threshold concentration in different bladders ranged from 0.01 to 0.1 μg/ml. The concentration producing a maximal response

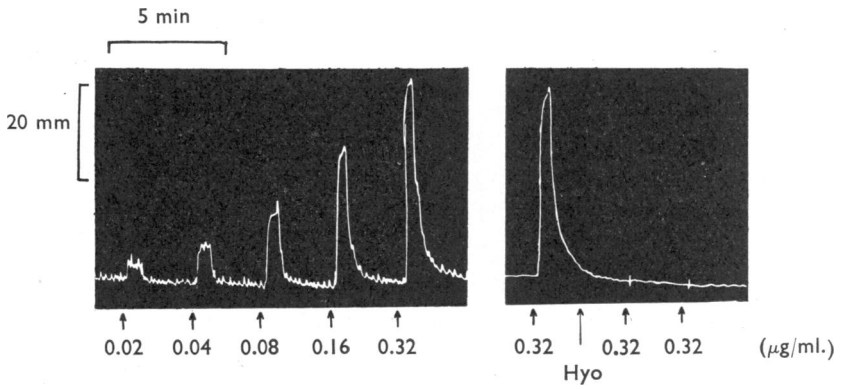

Fig. 1. Rat isolated urinary-bladder preparation. Acetylcholine (Ach) was added to the bath at the arrows in concentrations indicated (μg/ml.) for 30 sec. At Hyo, hyoscine (0.2 μg/ml.) was added to the bath. Time calibration, 5 min; kymograph calibration, 20 mm.

was not determined; it was in excess of 400 μg/ml. even in bladders responding to a low threshold concentration. The contraction was proportional to log concentration. The responses to acetylcholine remained reproducible for more than 6 hr.

Physostigmine in a concentration of 0.02 μg/ml. potentiated the responses to acetylcholine (Fig. 2). Higher concentrations of physostigmine (1 to 4 μg/ml.) caused a slow rise in tone of the bladder. After repeatedly washing the bath, the tone slowly returned to its initial level.

Atropine or hyoscine, in a concentration of 0.1 μg/ml., abolished the response to small doses of acetylcholine (Fig. 1). However, large doses of acetylcholine overcame the blockade (Fig. 6). The blockade to low doses persisted despite repeated washing.

Bradykinin. This caused a slow contraction of the bladder, taking about 1 min to develop fully. The response did not begin until about 10 sec had elapsed. After washing out the bradykinin, the bladder relaxed slowly (Fig. 2). In experiments with bradykinin, it was given at intervals of not less than 10 min to avoid tachyphylaxis, which was not seen. Physostigmine (0.02 μg/ml.) potentiated the response to bradykinin, but not to the same

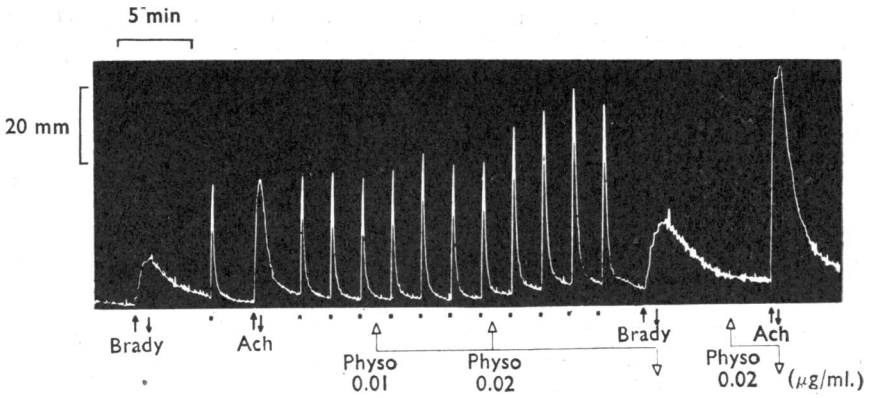

Fig. 2. The effect of physostigmine (Physo) on contractions of the rat bladder preparation produced by electrical stimulation (■), 0.04 μg/ml. of acetylcholine (Ach) and 0.1 μg/ml. of bradykinin (Brady). Drugs were placed into the bath at the upward arrows and washed out at the downward arrows. Electrical stimulation was with pulses of 1 msec duration at 1 shock/sec, for 10 sec at 2 min intervals. Time calibration, 5 min; kymograph calibration, 20 mm. All concentrations in μg/ml.

extent as that to acetylcholine (Fig. 2). Atropine or hyoscine, in concentrations just sufficient to block completely responses caused by small doses of acetylcholine, reduced responses to bradykinin by about one-half. Higher concentrations of atropine or hyoscine had no greater effect.

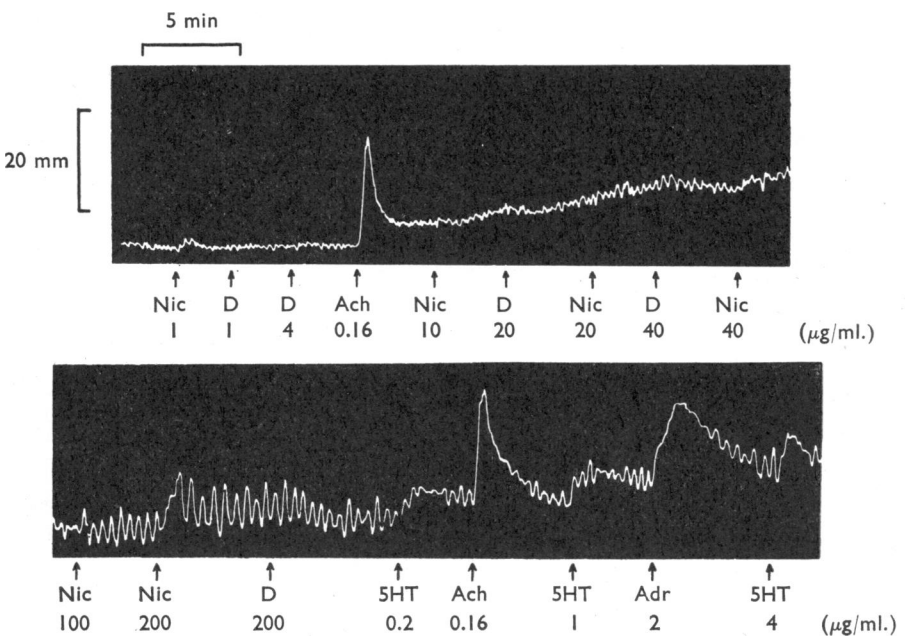

Fig. 3. Effects of nicotine (Nic), dimethylphenylpiperazinium (D), acetylcholine (Ach), 5-hydroxytryptamine (5HT) and adrenaline (Adr) on the rat isolated bladder preparation. Acetylcholine was present in the bath for 30 sec, the other drugs were present for 1 min. Time calibration, 5 min; kymograph calibration, 20 mm. All concentrations in μg/ml.

5-Hydroxytryptamine. In concentrations of 0.2 to 10 μg/ml., 5-hydroxytryptamine produced a slowly developing contraction of the bladder (Fig. 3). Morphine (10 μg/ml.) did not affect the response, but methysergide (0.25 μg/ml.) readily blocked it. These results suggest that 5-hydroxytryptamine acts on muscular receptors.

Catechol amines. Adrenaline and noradrenaline (0.1 to 2 μg/ml.) produced slow contractions (Fig. 3). The maximal response to adrenaline and noradrenaline was much smaller than the maximal response to acetylcholine. When the bladder was contracted in the presence of acetylcholine, adrenaline and noradrenaline produced relaxation. Isoprenaline (1 μg/ml.) invariably relaxed the bladder.

Ganglion stimulants. Dimethylphenylpiperazinium, in concentrations up to 200 μg/ml., had no effect (Fig. 3). Nicotine was ineffective in concentrations up to 100 μg/ml. but higher concentrations produced a small, slow contraction (Fig. 3).

Electrical stimulation

Electrical stimulation with trains of pulses caused an immediate rapid contraction of the bladder, with relaxation beginning at the end of stimulation. Typical records are shown in Figs. 2, 4 and 6. The pulse duration was threshold at 0.2 to 0.4 msec and optimal at 1 to 2 msec. The relationship between the frequency of pulses and contraction can be seen in Fig. 5 (middle curve) and Fig. 8 (upper curve). The threshold frequency was 0.5 to 1 shock/sec and the maximal response was obtained with 50 to 100 shocks/sec. Suitable submaximal contractions were elicited with 1 msec pulses at 10 or 20 shocks/sec applied for 10 sec at 2 min intervals. These conditions of stimulation were used in most experiments, and gave contractions which remained constant in size for several hours.

Effect of drugs on responses to electrical stimulation

Hexamethonium, in concentrations up to 100 μg/ml., did not affect the contractions produced by electrical stimulation (Fig. 4, *a*). Atropine, in a concentration of 0.1 μg/ml., reduced the contractions by about one-third. In the experiment shown in Fig. 4, *b*, higher concentrations of atropine, up to 10 μg/ml., had no further effect. The effects of hyoscine are illustrated in Fig. 6. A concentration of 0.05 μg/ml. reduced the heights of the contractions to an extent depending on the stimulus frequency; thus there was a greater reduction of responses to higher stimulus frequencies. In the presence of higher concentrations of

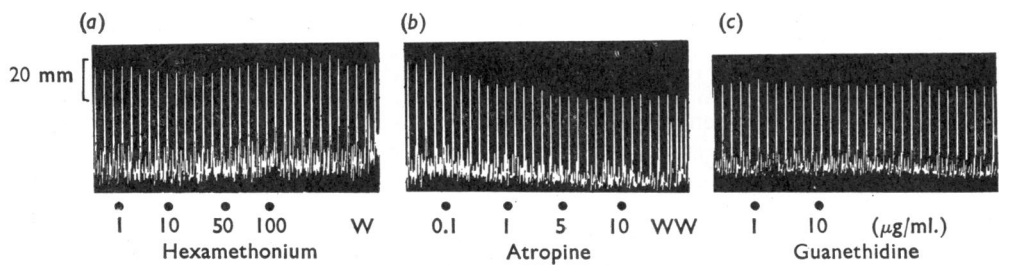

Fig. 4. Effect of drugs on responses of bladder preparation to electrical stimulation with pulses of 0.4 msec duration at 20 shocks/sec for 10 sec every 2 min. In (*a*) hexamethonium, in (*b*) atropine, and in (*c*) guanethidine were present in the concentrations indicated. At W the bath was washed. Kymograph calibration, 20 mm.

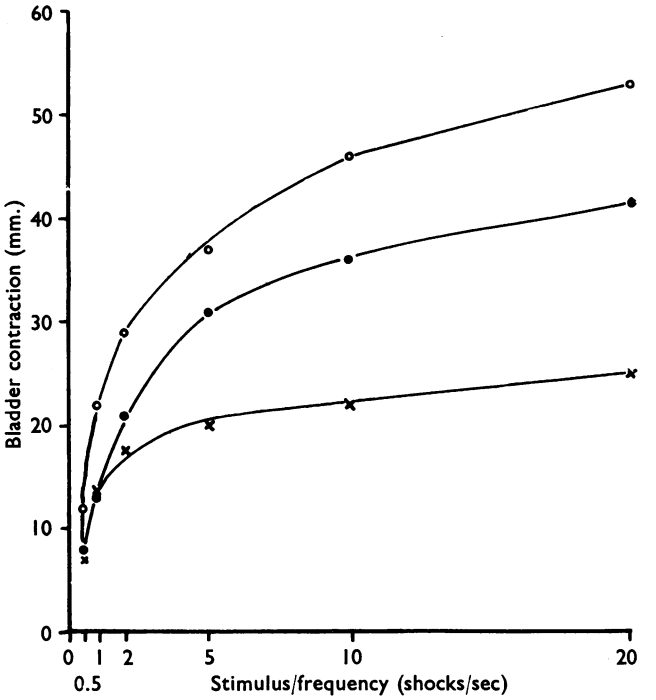

Fig. 5. Relationship between the contractions of the bladder preparation (ordinate, in mm on kymograph) and the stimulus frequency, with 1 msec pulses applied for 10 sec at 2 min intervals (abscissa, shocks/sec). Initial observations, ●——● ; observations after 0.02 μg/ml. of physostigmine, o——o ; after hyoscine, 0.1 μg/ml., ×——×.

hyoscine, up to 10 μg/ml., the responses to electrical stimulation with pulses at 1 and 2/sec were abolished and responses to higher stimulus frequencies were reduced.

Physostigmine (0.02 μg/ml.) potentiated the contractions (Fig. 2). Higher concentrations of physostigmine (1 μg/ml.) caused a spasm of the bladder, and stimulation then produced a potentiated contraction superimposed on the spasm; this contraction was maintained for some time after the end of the stimulation and was followed by a slow relaxation. Fig. 5 shows the effects of physostigmine (0.02 μg/ml.) in potentiating the contractions produced by various frequencies of stimulation. Then hyoscine (0.1 μg/ml.) added to the bath not only abolished the potentiation, but reduced the contractions to below their control heights.

Hemicholinium, in concentrations of 50 to 300 μg/ml., caused a gradual impairment of the responses in the course of about 3 hr during which regular stimulation was given. After the contractions had been completely blocked they were partly restored when the bath was washed and choline (200 μg/ml.) was added (Fig. 7). The blocking action of hemicholinium was more apparent at high stimulus frequencies. Fig. 8 shows the responses to several stimulus frequencies at the start of an experiment, and after repeated stimulation in the presence of hemicholinium.

Guanethidine (Fig. 4, *c*) or bretylium, in concentrations of 1 to 10 μg/ml., had no effect on the responses to electrical stimulation. Methysergide (1 μg/ml.) also failed to affect these

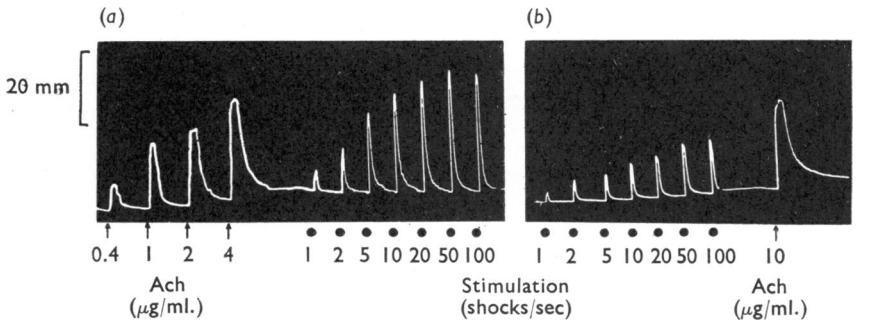

Fig. 6. Responses of the bladder preparation to electrical stimulation and to acetylcholine before (*a*) and after (*b*) hyoscine (0.05 μg/ml.). Acetylcholine (Ach) was given in the concentrations indicated (at the arrows), and the electrical stimulation, applied at ●, was with pulses of 2 msec duration in trains lasting 10 sec at 2 min intervals in the frequencies indicated (shocks/sec). Kymograph calibration, 20 mm.

responses. Morphine (10 μg/ml.) reduced the responses. Procaine (100 μg/ml.) abolished the response to stimulation.

The contractions of the bladder in response to electrical stimulation were greatly reduced by cooling to 19° C, or after turning off the oxygen supply to the bath.

Acetylcholine content of the bladder

Extracts of the rat bladder, prepared by the method of Beani & Bianchi (1963) for extracting acetylcholine, caused contraction of the guinea-pig ileum and a fall in blood pressure of the pithed rat. The contractions of the ileum persisted after diphenhydramine and after methysergid (1 μg/ml.), but were abolished by hyoscine (0.2 μg/ml.). Boiling the extracts for 5 min at about *p*H 8 destroyed their activity.

The activity of the extracts on the ileum, expressed as the acetylcholine content of the bladder, is given in Table 1. The activity of some extracts was also estimated from their effects on the blood pressure of the rat. There was reasonable agreement between the results obtained in parallel assays. Hyoscine (1 mg) abolished the depressor responses both

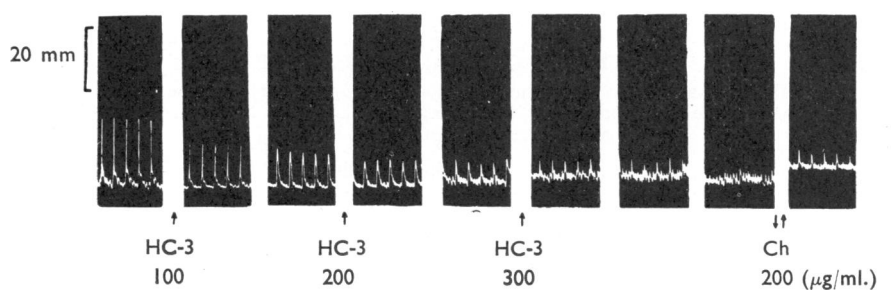

Fig. 7. Effect on the bladder preparation of hemicholinium (HC-3, in the concentrations indicated) in reducing responses to electrical stimulation with 2 msec pulses at 50 shocks/sec for 10 sec every 2 min. Between each panel, 15 min elapsed. At Ch, choline was added and partly restored the contractions. Kymograph calibration, 20 mm.

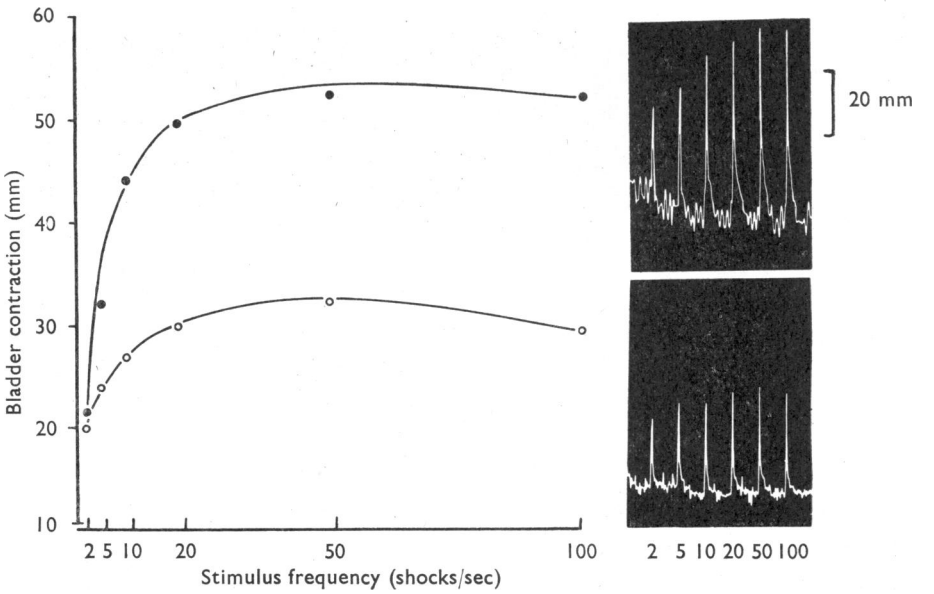

Fig. 8. Effect of hemicholinium on the responses of bladder preparation to various frequencies of stimulation. The graph shows on the ordinate the contractions in mm (on the kymograph) and on the abscissa the stimulus frequency in shocks/sec. ●——●, Initial observations; ○——○, after repeated stimulation with 2 msec pulses at 50 shocks/sec for 10 sec every 2 min in the presence of hemicholinium (200 μg/ml.) for 90 min. The tracings on the right are portions of the record before (above) and after (below) treatment with hemicholinium. Kymograph calibration, 20 mm.

to acetylcholine and to the extracts. Chang & Gaddum (1933) found that the urinary bladder of the dog had an acetylcholine chloride equivalent concentration of 1.2 μg/g.

In three experiments the bladders were set up in an organ-bath and stimulated electrically with 2 msec pulses at 50 shocks/sec for 15 sec every 2 min. The stimulation was continued in the presence of hemicholinium (50 μg/ml.) for 3 hr, when the contractions were less than one-half of their initial height, and then the bladders were extracted. The acetylcholine

TABLE 1

ACTIVITY OF BLADDER EXTRACTS

The activity is calculated as due to acetylcholine chloride in μg/g of fresh tissue. Values in parentheses refer to estimations on the blood pressure. * Electrical stimulation on one side. † Electrical stimulation applied simultaneously to both sides

Acetylcholine activity	
Normal bladder (μg/g)	After electrical stimulation in the presence of hemicholinium (μg/g)
0·7	0·7*
0·8	1·6†
1·7	2·6* (1·8)
2·4 (1·8)	
3·6	
4·1 (3·7)	

equivalent concentrations of the extracts are given in Table 1. These values were not different from those of bladders which had not been stimulated.

DISCUSSION

In mammals the vesical nerve branches from the pelvic plexus join the bladder at the point where the ureters enter (Langley & Anderson, 1895; Elliot, 1907), and according to Gruber (1933) these nerves are nonmyelinated. Bubić (unpublished observation) demonstrated histologically in the rat that nerve fibres accompany the ureter.

The results presented in this paper indicate that the contractions of the rat bladder, produced by electrical stimuli applied to the ureter, are due to excitation of nerves. The parameters of the stimuli are similar to those which excite autonomic nerve fibres to other organs. It is unlikely that the contractions were due to excitation propagated through the smooth muscle of the ureter to the bladder since the pulse width of rectangular shocks necessary for propagated excitation in the smooth muscle exceeds 20 msec (Bülbring, Burnstock & Holman, 1958). According to Bozler (1962) the urinary bladder, being a multi-unit smooth muscle, is activated predominantly by motor nerve impulses and shows no or little intramuscular conduction. The effect of procaine in blocking the contractions suggests also that the excitation is propagated by nerve fibres.

The responses of the bladder to stimulation of the vesical nerves were not affected by hexamethonium, which suggests that the nerves are postganglionic. Ganglion-stimulating drugs were without effect on the isolated bladder. These observations suggest that the rat bladder does not contain ganglia providing motor innervation. Ursillo & Clark (1956) found that most bladder strips from the rabbit were ganglion-free. However, Burnstock & Campbell (1963) found that ganglia were present in possum isolated bladder.

The responses to stimulation of the vesical nerves persisted in the presence of atropine or hyoscine, although the responses to added acetylcholine were abolished. The atropine-resistance of responses of the bladder to nerve stimulation is common to many species; it has been observed in the dog, cat and rabbit (Langley & Anderson, 1895; Henderson & Roepke, 1935; Ursillo & Clark, 1956; Ursillo, 1961; Edge, 1955) and in the possum (Burnstock & Campbell, 1963). These observations may indicate that the nerves were not cholinergic; however, Ambache (1955) was of the opinion that failure of atropine to block responses to nerve stimulation is not in itself proof that the nerves are noncholinergic. There is evidence that the nerves to the rat bladder are cholinergic. Thus physostigmine potentiated the responses. Physostigmine also potentiated responses of the bladder to stimulation of vesical nerves in the cat (Edge, 1955), rabbit (Ursillo & Clark, 1956) and possum (Burnstock & Campbell, 1963). Hemicholinium blocked the responses. Hemicholinium has been reported to block the synthesis of acetylcholine in the superior cervical ganglion of the cat and this block resulted in depletion of the ganglion store of acetylcholine and failure in transmission (MacIntosh, Birks & Sastry, 1956). In the present experiments, however, there was no depletion of acetylcholine from the bladder after partial failure produced by hemicholinium. An explanation may be that only a small fraction of the total acetylcholine is immediately available for release. Wong & Long (1961) have shown that hemicholinium blocked the contractions of the dog bladder elicited by stimulation of the pelvic (cholinergic) and the hypogastric (adrenergic) nerves. The latter effect could be

blockade of a cholinergic mechanism in the adrenergic nerves, the response of the effector cells being due to noradrenaline released secondary to acetylcholine, as suggested by Burn & Rand (1962).

It has been suggested that some or all of the parasympathetic nerves resistant to atropine are noncholinergic (Ursillo & Clark, 1956; Henderson & Roepke, 1935; Singh, 1964), and this may be an explanation for the atropine-resistance of the responses to vesical nerve stimulation in the rat.

Gruber (1933), in his review on the autonomic innervation of the genito-urinary system, states that " it is now absolutely certain that the autonomic innervation of the bladder comes from two sources, namely, the sympathetic . . . and the parasympathetic . . . each supplying both motor and inhibitor impulses." In the cat (Edge, 1955) and in the dog (Wong & Long, 1961; Garret, 1963), the sympathetic fibres to the bladder (derived from the hypogastric nerves) are adrenergic, and their stimulation causes contraction. However, it is unlikely that the atropine-resistant contractions to stimulating the vesical nerves in the rat are due to motor adrenergic fibres, because guanethidine and bretylium were without effect, and because noradrenaline and adrenaline failed to mimic the responses.

Another noncholinergic mechanism that has been invoked to explain atropine-resistance is the release of polypeptides. Thus, bradykinin has been demonstrated to be released after stimulation of the cholinergic nerves to the sweat glands (Fox & Hilton, 1958) and salivary glands (Hilton & Lewis, 1957). Singh (1964) reported that two polypeptides, termed " neurokinin," were released on stimulating the vagus nerves to the frog's stomach. Bradykinin did cause contraction of the rat bladder, and moreover the contraction was slightly reduced by atropine and enhanced by physostigmine. However, the response to bradykinin occurred only after a latent period and developed slowly. The effects of bradykinin on the bladder had the characteristics of its effects on some other smooth muscles (Elliott, Horton & Lewis, 1960; Konzett & Stürmer, 1960). It seems unlikely that bradykinin mediates the responses of the bladder to nerve stimulation. In fact, if part of the response to bradykinin were due to release of acetylcholine, this would explain the way in which its actions were altered by atropine and physostigmine. Weigerhausen, Stopp & Eichstädt (1964) reported that part of the contraction caused by bradykinin on the guinea-pig ileum was due to release of acetylcholine.

Another explanation for persisting responses to acetylcholine released from nerves was given by Dale & Gaddum (1930). Their explanation, designated " proximity theory " by Ambache (1955), was that acetylcholine was liberated in such an intimate relationship to the receptor mechanisms that atropine could not prevent its access. Ursillo & Clark (1956) suggested that receptors activated by nervously released acetylcholine were inaccessible to atropine. Our suggestion is that nerve endings to the bladder discharge their acetylcholine in such a way that the local concentration in the immediate vicinity of patches of receptors is sufficiently high to overcome competitively the blockade of lower concentrations of acetylcholine, produced by atropine or hyoscine. The acetylcholine diffusing away from these regions of high concentration would activate other free receptors, in the same way as does acetylcholine added from outside. It would be expected that more acetylcholine would diffuse through the tissue after nerve stimulation at higher frequencies. Our findings that hyoscine was more effective in blocking responses to higher frequencies supports this supposition.

SUMMARY

1. Electrical stimulation of the vesical nerves adjoining the ureter caused contractions of the isolated urinary bladder of the rat. These contractions were unaffected by hexamethonium, potentiated by physostigmine and partially reduced by atropine or hyoscine.

2. The contractions produced by repeated stimulation were gradually blocked by hemicholinium in the course of 2 to 3 hr, and this block was partially reversed by choline.

3. Bradykinin, 5-hydroxytryptamine, adrenaline and noradrenaline produced slow increases in tone of the rat isolated bladder. Ganglion stimulants had no effect, suggesting that the bladder contains no autonomic ganglia.

4. Acetylcholine produced rapid contractions as large as those to nerve stimulation. An acetylcholine-like substance was found in extracts from the bladder.

5. It is concluded that the vesical nerves accompanying the ureter contain postganglionic cholinergic fibres. It is suggested that the atropine-resistant responses to nerve stimulation are due to the competitive reversal of atropine blockade by high local concentrations of acetylcholine released from nerve terminals.

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