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THE ATROPINE-RESISTANCE OF THE RESPONSE TO INTRINSIC NERVE STIMULATION OF THE GUINEA-PIG BLADDER

BY

G. B. CHESHER AND R. H. THORP

From the Department of Pharmacology, University of Sydney, Sydney, Australia

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The urinary bladder responds to parasympathetic nerve stimulation by contraction but it has been shown in several species that this response is not blocked by atropine. Acetylcholine applied to isolated bladder preparations, on the other hand, produces a contraction which is abolished by atropine in the manner characteristic of a muscarinic response.

As early as 1911, Langley observed this anomalous behaviour of the bladder in the dog, cat and rabbit but until recently studies of this phenomenon in other species have been few. Burnstock & Campbell (1963) and Burnstock, O'Shea & Wood (1963) have described its occurrence in the ring-tailed possum (*Pseudocheirus peregrinus*) and the toad (*Bufo marinus*). Carpenter (1963) also noted the same results for the bladder of the rat.

This paper describes studies on the response of the isolated bladder of the guinea-pig to electrical stimulation and to application of drugs.

METHODS

Adult guinea-pigs of either sex, weighing 250 to 400 g, were killed by a blow on the head. Both ureters were tied and cut and the bladder was flushed out with 1 to 2 ml. of Locke solution through a cut in the urethra. A glass cannula was then inserted into the bladder through the urethra, tied into place and filled with Locke solution to distend the bladder. The cannulated bladder was immersed in the organ-bath (25 ml. capacity) filled with Locke solution and bubbled with 95% oxygen and 5% carbon dioxide. The cannula was connected to a U-tube containing black ink and the intraluminal pressure under these conditions was 60 to 80 mm of water. One arm of the U-tube passed through a close-fitting sleeve in which length-wise slits had been cut on opposite sides. The sleeve was illuminated by a lamp and the light passing through the slit was allowed to fall on a selenium photoelectric cell. Movement of the bladder displaced the fluid in the cannula, varying the level of the ink of the U-tube and so changing the illumination of the photo-cell. The potential changes so produced were recorded directly on a self-balancing potentiometric recorder. For electrical stimulation one platinum electrode was inserted through the cannula into the lumen of the bladder and another, which was made the anode, was placed directly in the organ-bath. The shocks were rectangular, submaximal (2 to 5 V) or supramaximal (20 V), and of 0.5 to 1 msec duration. The stimulus frequency was varied. The preparation showed considerable spontaneous activity, although this could be reduced by keeping the bath temperature at 30° C.

Treatment of guinea-pigs with reserpine. Reserpine was dissolved in a 20% solution of ascorbic acid and a dose of 10 mg/kg was given intraperitoneally on four successive days, the animal being used on the fifth day. During this treatment the animals were kept in a warm room.

The following drugs were used and the concentrations are expressed as weight of the salts per unit volume: acetylcholine chloride, adrenaline tartrate, atropine sulphate, bretylium tosylate (Burroughs Wellcome), physostigmine sulphate, histamine acid phosphate, 5-hydroxytryptamine creatinine phosphate, isopropamide iodide (Smith Kline & French), muscarine iodide, nicotine hydrogen tartrate, noradrenaline bitartrate, procaine hydrochloride and reserpine.

RESULTS

Response to electrical stimulation

The preparation responded by contraction to transmural stimulation with the cathode inside the bladder. The threshold to stimulation with pulses of 0.5 msec duration was approximately 2 V and a maximal response could be elicited by 5 to 15 V. The response was considered to be the result of excitation of nerves and not a direct effect on the muscle because it was abolished by a number of techniques which produce physiological denervation, such as hypoxia produced by bubbling the organ-bath with 95% nitrogen and 5% carbon dioxide (Garry, 1928; Day & Vane, 1963), storage at 1 to 5° C for 3 days (Vogt, 1943; Ambache, 1955), or cooling to 17° C (Innes, Kosterlitz & Robinson, 1957; Day & Vane, 1963), although in this instance abolition was incomplete. In all of these circumstances the response to acetylcholine (4×10^{-8} g/ml.) was unchanged or potentiated, providing evidence for the integrity of the muscle. The response to electrical excitation was potentiated by physostigmine (10^{-7} g/ml.), reduced by hexamethonium (8×10^{-5} g/ml.) and blocked by procaine (10^{-4} g/ml.).

A contraction could be obtained to single pulses, though a maximal response required a train of stimuli. At 30° C a maximal response was obtained with a stimulus frequency of 16 to 20 shocks/sec, with supramaximal stimuli (Fig. 1). The shape of the stimulus frequency/response curve was unchanged by bretylium (4×10^{-5} g/ml.) or by previous treatment of the animal with reserpine. It was noted, however, that the response of the reserpine-treated bladder was greatly potentiated.

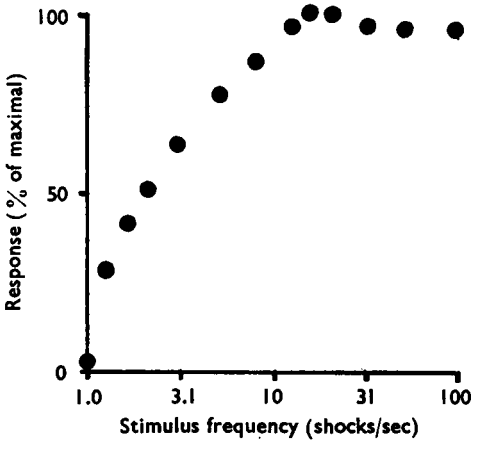

Fig. 1. The effect of varying the stimulus frequency on the response of the guinea-pig bladder. Each point is the mean of three observations, expressed as a percentage of the maximal response. Stimuli were supramaximal (20 V), 1 msec duration, and applied for 5 sec at 5-min intervals.

Response to drugs

The bladder contracted when exposed to suitable concentrations of nicotine (3×10^{-6} g/ml.), acetylcholine (4×10^{-8} g/ml.), muscarine (8×10^{-9} g/ml.), 5-hydroxytryptamine (8×10^{-7} g/ml.) or histamine (8×10^{-7} g/ml.). The most active of these drugs was muscarine, which was approximately five-times as active as acetylcholine. The log dose/response relationship for acetylcholine was linear between 8×10^{-8} and 8×10^{-9} g/ml.

The contraction due to muscarine was slow in onset and recovered more slowly to the resting state than a contraction after nicotine or acetylcholine. The contraction due to nicotine was much faster and of shorter duration, while the " muscarinic " effect of acetylcholine lay between those of muscarine and nicotine. The " nicotinic " response to acetylcholine, produced by a much larger dose in the presence of atropine, was indistinguishable from that due to nicotine itself. These results are shown in Fig. 2.

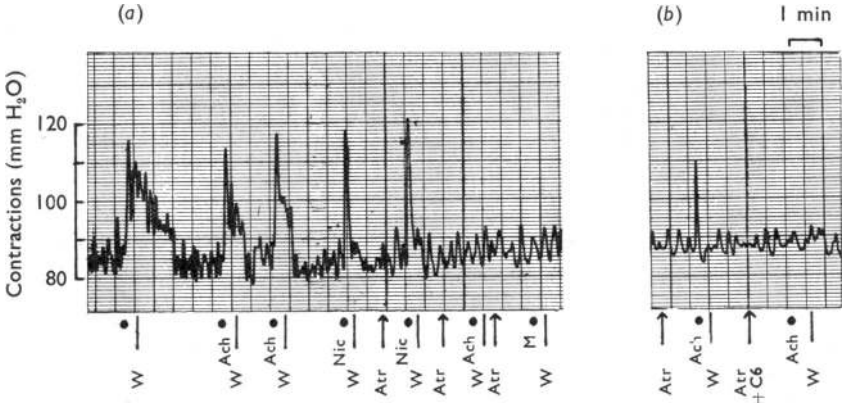

Fig. 2. The effect of atropine upon the responses produced by the addition to the bath of acetylcholine, muscarine and nicotine. In (*a*), the response to nicotine is unaffected by a concentration of atropine which abolishes the responses both to acetylcholine and to muscarine; (*b*) in the presence of atropine, acetylcholine in higher concentration produces a contraction of the bladder muscle which is blocked by hexamethonium. M = muscarine, 8×10^{-9} g/ml.; Ach = acetylcholine, 4×10^{-8} g/ml. in (*a*) and 4×10^{-6} g/ml. in (*b*); Nic = nicotine, 3×10^{-6} g/ml.; C6 = hexamethonium, 8×10^{-5} g/ml.; Atr = atropine, 3.2×10^{-8} g/ml.; W = wash.

An indication of the mode of action of acetylcholine and nicotine on the bladder was obtained by the " denervation " techniques previously described. When the response of the bladder to electrical stimulation had been abolished by refrigeration at 1 to 5° C for 3 days or by hypoxia, it also failed to respond to nicotine, although still responding to acetylcholine as before. Cooling to 17° C potentiated the response to acetylcholine though it greatly reduced the responses to nicotine and electrical stimulation.

Adrenaline and noradrenaline, in the majority of preparations, were without effect in concentrations up to 4×10^{-7} g/ml. In a few preparations relaxation of tone was observed with no change in the level of spontaneous rhythmicity.

Effect of atropine

Concentrations of atropine which completely blocked the response to acetylcholine and muscarine showed no antagonism of the response produced by nicotine. When the " muscarinic " effect of acetylcholine was blocked by atropine, the " nicotinic " effect was seen after increasing the concentration of acetylcholine one hundred times (Fig. 2).

The " nicotinic " response to acetylcholine, like that to nicotine itself, was blocked by hexamethonium at a concentration that did not affect the " muscarinic " response to acetylcholine (Figs. 2 and 3).

The response of the bladder to electrical stimulation also was resistant to block by atropine (Fig. 4) in concentrations as high as 2×10^{-6} g/ml. The first response produced after the addition of atropine (3.2×10^{-8} g/ml.) to the bath was potentiated, an effect also noted with nicotine. The synthetic antimuscarinic drug isopropamide also failed to block the response either to electrical stimulation or to nicotine, but attempts with several concentrations of isopropamide (3.2×10^{-8} to 4×10^{-6} g/ml.) to reproduce the potentiation seen with atropine were without success. The concentrations of atropine and isopropamide were sufficient completely to block the response to externally applied acetylcholine.

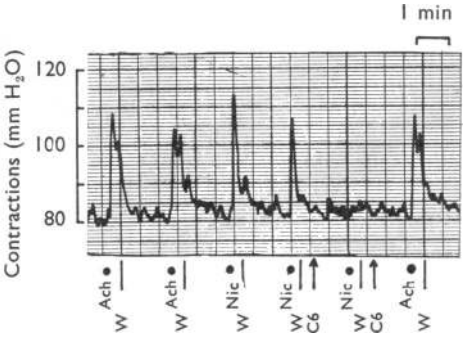

Fig. 3. The effect of hexamethonium upon the responses produced by acetylcholine and nicotine. Ach = acetylcholine, 4×10^{-8} g/ml.; Nic = nicotine, 3×10^{-6} g/ml.; C6 = hexamethonium, 4×10^{-5} g/ml.; W = wash.

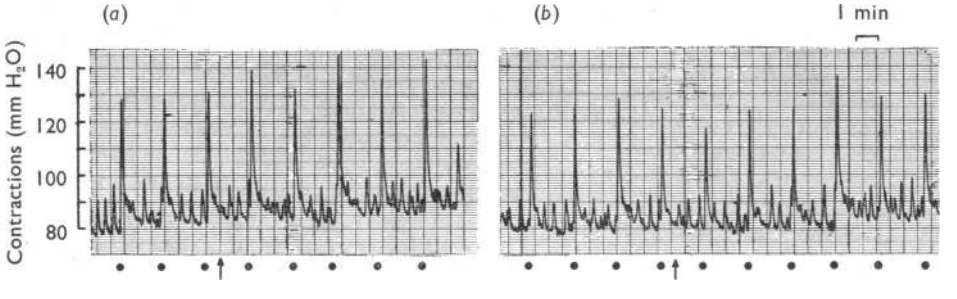

Fig. 4. The effect of atropine upon the responses produced by electrical stimulation. ● = 40 shocks at a frequency of 20 per sec, 15 V, 1 msec. At the arrows, Locke solution was changed to one containing atropine: (*a*) 3.2×10^{-8} g/ml.; (*b*) 2×10^{-6} g/ml.

The effect of hexamethonium and tubocurarine

Ganglionic blockade by hexamethonium (4×10^{-5} g/ml.) abolished the response to nicotine and reduced that to electrical stimulation by approximately 10%. A similar reduction was produced by tubocurarine (4×10^{-5} g/ml.). However, if ganglion-block had been produced by hexamethonium, the subsequent addition of tubocurarine produced no further reduction in the response to electrical stimulation.

The effect of physostigmine

Physostigmine (10^{-7} g/ml.) potentiated the response of the bladder to electrical stimulation both of maximal (20 V) and of submaximal (2 to 5 V) strength, and with stimulus frequencies of 5 or 20 shocks/sec. The responses to nicotine and acetylcholine were also potentiated by physostigmine (Fig. 5). With physostigmine still in the bath, the potentiated responses were reduced by atropine, and those to acetylcholine were blocked. However, the block by atropine of the responses to nicotine and electrical stimulation was confined only

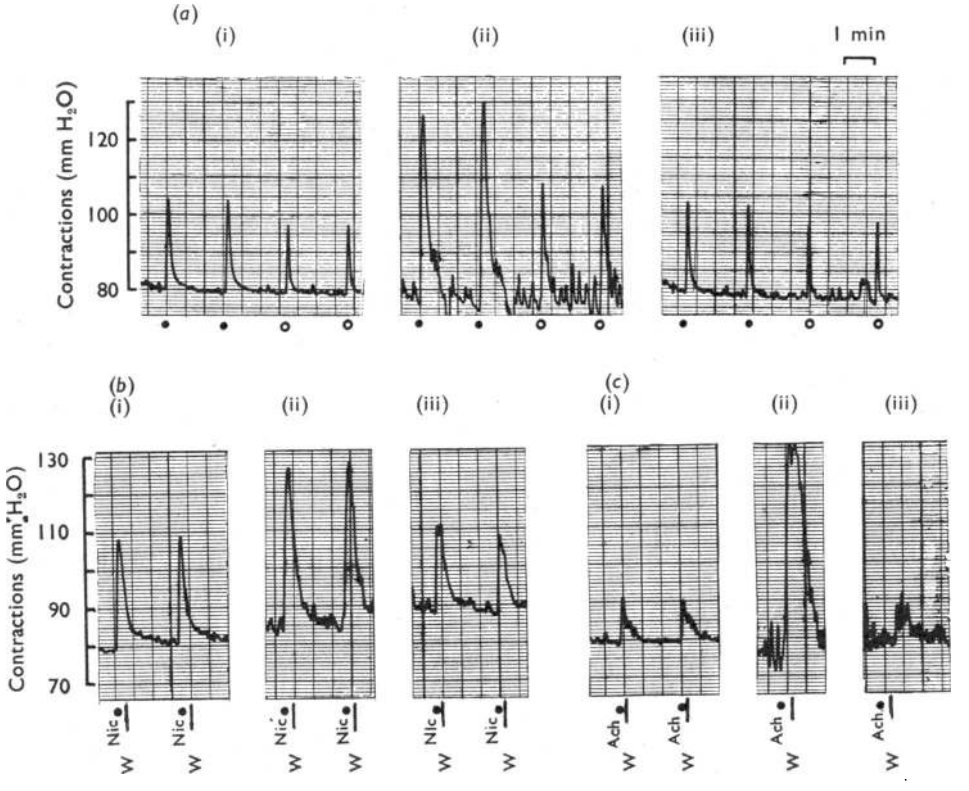

Fig. 5. The potentiation by physostigmine of the response of the bladder to (*a*) electrical stimulation, (*b*) nicotine and (*c*) acetylcholine, and the abolition of the potentiated portion of the response by atropine. (i) Before physostigmine; (ii) with physostigmine, 10^{-7} g/ml.; (iii) with physostigmine, 10^{-7} g/ml., and atropine, 3.2×10^{-8} g/ml. ● = 40 shocks at a frequency of 20 per sec, 2 V and 1 msec; ○ = 10 shocks at a frequency of 5 per sec, 2 V, 1 msec; Nic = nicotine, 3×10^{-6} g/ml.; Ach = acetylcholine, 4×10^{-6} g/ml.; W = wash.

to the potentiated portion of the response, the atropine-resistant portion remaining as before potentiation by physostigmine. This concentration of atropine, as has previously been shown, did not reduce the response to either nicotine or transmural stimulation, in the absence of physostigmine.

DISCUSSION

As the response of the bladder to electrical stimulation is blocked by the " physiological denervation " procedures described, and is reduced by hexamethonium and potentiated by physostigmine, it is concluded that the stimulation is of nervous tissue and is partly preganglionic and partly cholinergic.

The effects of bretylium and of treatment of the animal with reserpine upon the shape of the stimulus frequency/response curve of the guinea-pig bladder do not suggest a role for adrenergic fibres in the contraction produced. The absence of a contractile response to noradrenaline supports this view. Indeed, the stimulation characteristics resemble those for the parasympathetic fibres of the isolated colon and ileum of the rabbit (Garry & Gillespie, 1955; Day & Rand, 1961).

The fact that the response to externally applied acetylcholine is effectively blocked by atropine whilst that to electrical stimulation is not suggests that different receptors may be involved. The potentiation of the nervous response by physostigmine indicates that acetylcholine is involved, and moreover this acetylcholine is acting upon receptors which are blocked by atropine. Previous workers have shown that the bladders of other species show some sensitivity to atropine, but there is a stage where increasing the atropine concentration produces no further block. Ursillo (1961) and Ursillo & Clark (1956) have shown that this atropine-sensitive portion of the response was very much the same as other atropine-sensitive mechanisms. It seems therefore that two mechanisms might be involved in the response of the bladder to parasympathetic nerve stimulation. One of these mechanisms is certainly cholinergic as it is potentiated by physostigmine and blocked by atropine whilst the other is atropine-resistant and may or may not be cholinergic.

Dale & Gaddum (1930) suggested that the proximity of the nerve ending to the muscle was too close for the atropine molecule to gain access to the receptor. Such a close association would permit a very high local concentration of transmitter, which by competition would prevent access of the atropine to the receptor. With this concept the effect of physostigmine would be to allow some acetylcholine to " overflow " to other receptors where its concentration would be more favourable for the competitive antagonism by atropine. In this way, the " overflow " may constitute the atropine-sensitive mechanism described above.

SUMMARY

1. The isolated urinary bladder of the guinea-pig contracts to transmural electrical stimulation. This effect is believed to be due to stimulation of nerves and not to a direct effect upon the muscle.

2. The relationship of stimulus frequency to response of the bladder before and after bretylium, and in normal and reserpine-treated animals, provides no evidence for the involvement of sympathetic fibres in the contraction produced.

3. The bladder contracts to acetylcholine, muscarine and nicotine. The response to electrical stimulation is reduced and that to nicotine is abolished by hexamethonium in concentrations which have no effect on the response to acetylcholine. Concentrations of atropine sufficient to block the response to acetylcholine or muscarine have no effect on those to nicotine or electrical stimulation.

4. The responses to electrical stimulation and to nicotine are potentiated by physostigmine and the potentiated portion of the response is abolished by atropine.

5. It is suggested that two mechanisms are involved in the response of the guinea-pig bladder to stimulation of its intrinsic nerves. One mechanism is cholinergic, the other is atropine-resistant and may or may not be cholinergic.

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