J. Pharm. Pharmac., 1966, **18**, 417–423 Received January 25, 1966

The "nicotinic" and "muscarinic" receptors of the urinary bladder of the guinea-pig

G. B. CHESHER AND BARBARA JAMES

The action of acetylcholine and nicotine on the urinary bladder of the guinea-pig has been examined using three techniques of physiological denervation. Nicotine was neurogenic and equiactive concentrations of acetylcholine were myogenic. Whilst acetylcholine has both "muscarinic" and "nicotinic" effects on the guinea-pig bladder, the concentration required to stimulate the nicotinic receptors was approximately 100 times that required for the muscarinic receptors. These results indicate that the nicotinic receptors are confined to nervous tissue, and do not support the suggestion that there might be non-neuronal nicotinic receptors in the bladder musculature of the guinea-pig. The possibility of a non-cholinergic component in the post-ganglionic parasympathetic fibres to the bladder should still be considered.

THE isolated urinary bladder of the guinea-pig contracts to transmural electrical stimulation and to suitable concentrations of nicotine, acetylcholine or muscarine. The response to acetylcholine or muscarine can be completely abolished by atropine, but that to nicotine or transmural stimulation is resistant to muscarinic blockade (Chesher & Thorp, 1965). This phenomenon of atropine resistance to parasympathetic nerve stimulation in the urinary bladder has been observed in a number of species; it has been reported in the dog, the cat and the rabbit (Langley & Anderson, 1895; Henderson & Roepke, 1935; Edge, 1955; Ursillo & Clark, 1956; Ursillo, 1961); in the possum and the toad (Burnstock & Campbell, 1963; Burnstock, O'Shea & Wood, 1963); and in the rat (Carpenter, 1963; Huković, Rand & Vanov, 1965). These studies have provided evidence that a cholinergic component is involved in the response to nervous stimulation. The possibility of the involvement of a non-cholinergic, atropine-resistant component has also been suggested (Henderson & Roepke, 1934; Singh, 1964; Chesher & Thorp, 1965).

Gyermek (1961) examined the cholinergic blockade of cholinomimetic drugs on the bladder *in situ* of the dog and the cat, and showed the response to acetylcholine to have a significant ganglionic component. He suggested that the parasympathetic effector sites of the bladder may differ functionally from those of other organs. He postulated the presence of 'nicotinic' receptors in the bladder musculature and on these grounds considered that the assumption of a non-cholinergic component of the parasympathetic nerves of the bladder was improbable. However, if these atropine-resistant receptors are confined to nervous tissue, the possibility of a non-cholinergic component in the post-ganglionic fibres still exists.

The present work was undertaken to see if the response of the guinea-pig bladder *in vitro* shows a significant ganglionic component in its response to acetylcholine, and to examine the evidence for the presence in this species of non-neuronal "nicotinic" receptors in the bladder musculature.

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

Experimental

Guinea-pigs of either sex were killed by a blow on the head and bled. Both ureters were tied and cut and a glass cannula was inserted into the bladder via a cut in the urethra and tied into place. Locke solution was introduced into the cannula to distend the bladder which, in turn, was bathed in Locke solution, in an organ bath of 25 ml capacity, aerated with oxygen containing 5% carbon dioxide. The Locke solution was maintained at 30° unless otherwise stated, and was of the following composition: NaCl, 9·0; KCl, 0·42; $CaCl_2$, 0·24; $NaHCO_3$, 0·3; glucose, 1·0; g/litre. The method of recording was as previously described (Chesher & Thorp, 1965). Under these conditions the intraluminal pressure of the resting bladder was approximately 60 to 80 mm water.

Electrodes for electrical stimulation were placed in the Locke solution, one in the solution in the organ bath, the other in the solution within the lumen of the bladder. Square wave pulses of supramaximal amplitude (20 V) and 2 msec duration were delivered at a frequency of 20/sec.

Dose-response curves for acetylcholine and nicotine were made before and after physiological denervation by the techniques described below. The regression coefficient for each dose-response curve was calculated by the method of Burn, Finney & Goodwin (1950), and the results expressed in terms of the dose ratio (Gaddum, Hameed, Hathaway & Stephens, 1955). The dose ratio is the ratio of equiactive concentrations of the stimulant drug before and after the physiological denervation. For this calculation the concentration of stimulant drug required to produce a response of 40 mm water above the base line was chosen, this being approximately 50% of the maximal contraction under the conditions of these experiments.

"PHYSIOLOGICAL DENERVATION" TECHNIQUES

(*a*) Denervation by storage in the cold (Vogt, 1943; Ambache, 1946; Emmelin & Feldberg, 1947). The preparation was stored in Locke solution for 2 to 5 days at a temperature of 4–5°. After this time it was set up as previously described, at 30°.

(*b*) Denervation by cooling (Innes, Kosterlitz & Robinson, 1957; Gillespie & Wishart, 1957; Beleslin & Varagić, 1958). The temperature of the Locke solution bathing the bladder was reduced to 15°.

(*c*) Denervation by anoxia (Gross & Clark, 1923; Garry, 1928; Prasad, 1935; West, Hadden & Farah, 1951). The gas mixture bubbling through the solution in the organ bath was changed to one containing nitrogen, 95% and carbon dioxide, 5%.

After treatment by each of these techniques an equilibration period of 2 hr was allowed before any drugs were applied to the bladder. *Drugs used* were acetylcholine chloride, nicotine hydrogen tartrate, hexamethonium bromide and atropine sulphate. Doses are expressed as the salt.

Results

DENERVATION BY STORAGE IN THE COLD

In all experiments, storage of the bladder at 4–5° for 2 to 5 days completely abolished the response to electrical stimulation. The denervated bladder responded to acetylcholine though in most cases the dose-response curve was moved to the right. However, in one experiment (CS2) the response to acetylcholine was unaffected by the denervation and in another (CS4) the dose ratio was only 2·5. The response to nicotine, on the other hand, was abolished in all instances.

There were also qualitative differences in the response of the denervated bladder to acetylcholine. The time for the beginning of the contraction after the acetylcholine had been added to the bath was delayed, and both the speed of the response and the time for recovery after the drug was washed from bath were slower than before denervation.

The results expressed as dose ratios are given in Table 1, and the dose-response curves for experiment CS2 are shown in Fig. 1.

TABLE 1. THE RATIO OF EQUIACTIVE DOSES OF ACETYLCHOLINE AND NICOTINE AFTER/BEFORE PHYSIOLOGICAL DENERVATION BY STORAGE IN THE COLD

Experiment No.	Dose ratios		Storage time (days)
	Acetylcholine	Nicotine	
CS1	27·0	no response	5·0
CS2	1·0	,, ,,	1·5
CS3	17·4	,, ,,	4·5
CS4	2·5	,, ,,	3·5
CS5	39·0	,, ,,	5·0
CS6	28·2	,, ,,	3·5

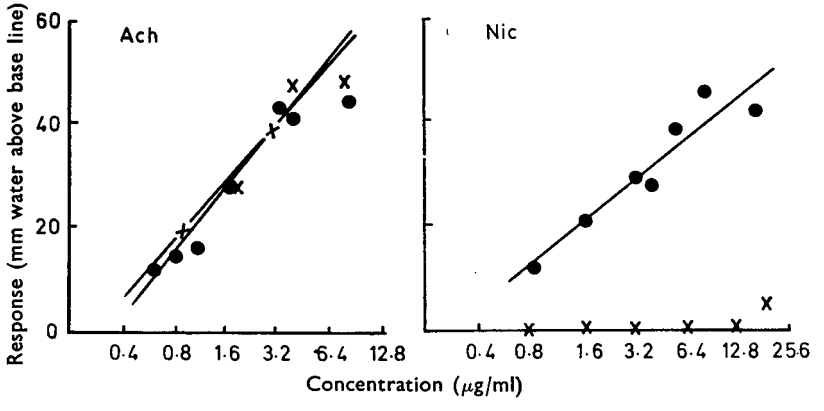

FIG. 1. Guinea-pig bladder. Log dose-response relationship to acetylcholine and nicotine, before (●——●) and after (×——×) physiological denervation by storage in the cold.

DENERVATION BY COOLING

Lowering the temperature of the bath fluid produced an increase in the tone of the bladder which often took more than 2 hr to return to the

baseline. Under the conditions of this experiment it was not possible to completely abolish the response of the bladder to transmural stimulation by cooling to 15°, though in all instances the response was reduced to less than 10% of that at 30°. The response to acetylcholine, though much slower, was little affected by cooling, whilst that to nicotine was significantly reduced, and in two instances was virtually abolished (See Fig. 2 and Table 2, exp. C2 and 4).

The dose ratios are given in Table 2 and the dose-response curves for Experiment C2 are shown in Fig. 2.

TABLE 2. THE RATIO OF EQUIACTIVE DOSES OF ACETYLCHOLINE AND NICOTINE AFTER/BEFORE PHYSIOLOGICAL DENERVATION BY COOLING AND ANOXIA

Experiment No.	Dose ratios	
	Acetylcholine	Nicotine
Cooling:		
C1	1·4	4·8
C2	1·7	*
C3	2·7	4·0
C4	2·1	*
C5	0·7	1·8
Anoxia:		
A1	1·4	9·8
A2	1·0	2·3
A3	0·7	*
A4	2·5	*
A5	1·0	*
A6	0·5	2·8
A7	32·5	*

* Only a small response (less than 20 mm H_2O above base line) could be obtained, and this did not show a dose-response relationship.

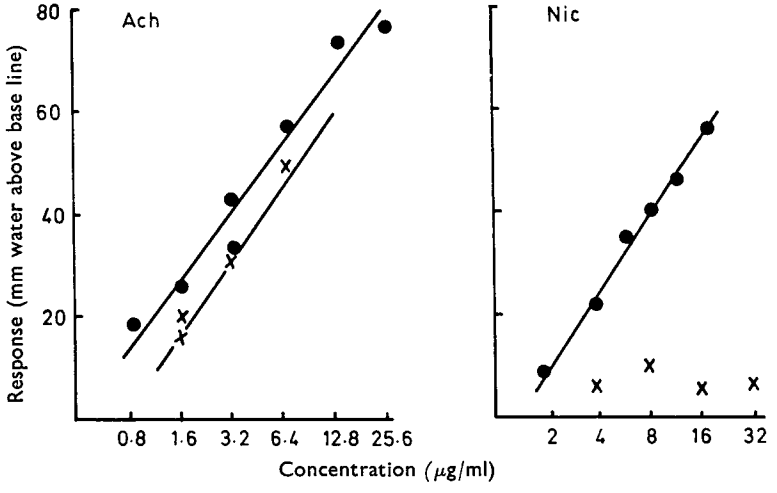

FIG. 2. Guinea-pig bladder. Log dose-response relationship to acetylcholine and nicotine, before (●——●) and after (×——×) physiological denervation by cooling to 15°.

DENERVATION BY ANOXIA

After exposure to the nitrogen-carbon dioxide for 2 hr, the response of the bladder to transmural stimulation was abolished in four experiments and reduced to less than 10% of the pre-treatment response in three (experiments A1, 2 and 6).

The response to acetylcholine was very little affected quantitatively except in experiment A7, though qualitatively the contraction was slower to begin and took longer to reach its peak.

In experiments A3, 4, 5 and 7, where denervation was complete, the response to nicotine was abolished. The preparations which gave a small response to transmural stimulation (experiments A1, 2 and 6) also responded to nicotine, though the dose-response curve was displaced to the right.

The results expressed as dose ratios and the dose-response curves for experiment A4, are shown in Table 2 and Fig. 3, respectively.

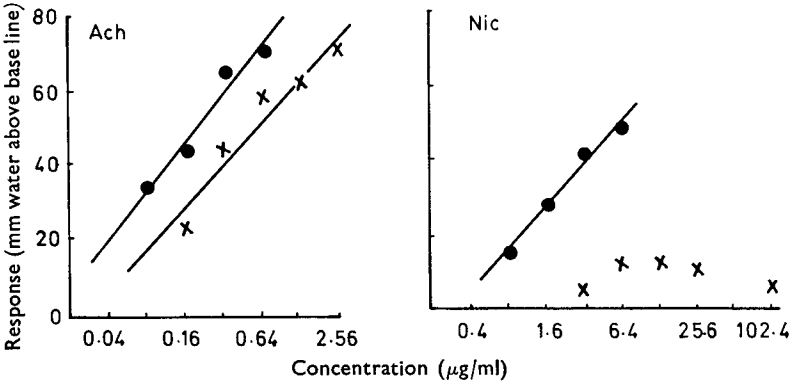

FIG. 3. Guinea-pig bladder. Log dose-response relationship to acetylcholine and nicotine, before (●——●) and after (×——×) physiological denervation by anoxia.

THE EFFECT OF ATROPINE

The atropine-resistant response of the guinea-pig bladder to nicotine permitted a clear demonstration of the "nicotinic" effect of acetylcholine (Chesher & Thorp, 1965). The "muscarinic" effect of acetylcholine was effectively abolished by atropine, though in the presence of this block an increase in the concentration of acetylcholine again produced a response. This contraction was not affected by further additions of atropine, though it was abolished by hexamethonium.

To determine the ratio of "nicotinic": "muscarinic" activity of acetylcholine on the preparation, the following experiment was made.

Equiactive concentrations of acetylcholine and nicotine were selected and, after the addition of atropine (0·16 μg/ml), the concentration of acetylcholine needed to produce a response similar to that produced by nicotine was determined.

In three experiments the acetylcholine concentration had to be increased one hundred times to produce a response which matched that induced by

nicotine. These responses to nicotine, and to acetylcholine in the presence of atropine, were abolished by hexamethonium (40 μg/ml).

Discussion

The denervation techniques we have employed are considered to produce a progressive inactivation of tissue, with the smooth muscle being the most resistant, the post-ganglionic fibre less so and the pre-ganglionic fibre being the most sensitive (Vogt, 1943; Gillespie & Wishart, 1957).

In all the experiments, acetylcholine showed the most resistance to the treatment given, and in a number of instances the response was unchanged or potentiated. In those experiments where the treatment reduced the response to acetylcholine, two possibilities must be considered.

It could be an indication of a nervous component in the response, or it could be due to an effect on the smooth muscle itself. In view of the small dose ratio we found for acetylcholine, which in some experiments was one or less, we consider that the reduced response to acetylcholine was due to an effect of the treatment on the muscle. These results indicate therefore that the response of the isolated bladder of the guinea-pig to acetylcholine is the result of a direct effect on the muscle and does not include a significant ganglionic component. Indeed, much higher concentrations of acetylcholine were needed before an indirect effect was obtained as was shown by the high ratio of "nicotinic" to "muscarinic" effects. In the presence of an atropine block of the muscarinic receptors, the concentration of acetylcholine had to be increased one hundred times before it elicited an equiactive "nicotinic" response. We would have expected this dose ratio to be much lower if acetylcholine, in the concentrations used in the absence of atropine, stimulated ganglion cells.

Further evidence for a purely muscarinic action of acetylcholine at these concentrations has been provided by the observation of Chesher & Thorp (1965) that concentrations of hexamethonium which abolished the response to nicotine had no effect on equiactive concentrations of acetylcholine.

The response to nicotine was sensitive to denervation. In all preparations where the response to transmural stimulation had been abolished, that to nicotine had also been blocked. In those experiments where denervation was not complete, and a small response to transmural stimulation remained, the dose-response curve to nicotine was displaced to the right.

In two experiments no response to nicotine could be elicited even though a small response to transmural stimulation remained. This effect could be explained on the basis of the progressive nature of the denervation. In these instances, the ganglion cells had been rendered insensitive to stimulation by nicotine whilst the post-ganglionic fibres were still responsive, to some degree, to transmural stimulation.

These results indicated that the response of the bladder to nicotine was indirect and was due to the stimulation of the autonomic ganglion cells. Ganglion cells do occur in the bladder musculature of some species

(Gruber, 1933) and we have observed their presence in the guinea-pig bladder, when stained with methylene blue. Apart from the receptors of the intramural ganglion cells, there was no evidence for the presence of nicotinic receptors in the bladder musculature of the guinea-pig. Should such receptors exist, and assuming them to be resistant to the effects of denervation (as were the muscarinic receptors), one would expect that a response to nicotine could still be elicited after denervation. As we found that this was not so, we conclude that the nicotinic receptors in the guinea-pig bladder are confined to the ganglion cells.

The possibility, therefore, that the atropine-resistant response to stimulation of the postganglionic parasympathetic fibres of the bladder might be due to the involvement of a non-cholinergic transmitter, must still be considered.

References

Ambache, H. (1946). *J. Physiol., Lond.*, **104**, 226–287.
Beleslin, D. B. & Varagić, V. (1958). *Br. J. Pharmac. Chemother.*, **13**, 226–270.
Burn, J. H., Finney, D. J. & Goodwin, L. G. (1950). *Biological Standardization*, 2nd ed., p. 51, London: Oxford University Press.
Burnstock, G. & Campbell, G. (1963). *J. exp. Biol.*, **40**, 421–436.
Burnstock, G., O'Shea, J. & Wood, M. (1963). *Ibid.*, **40**, 403–419.
Carpenter, F. G. (1963). *Am. J. Physiol.*, **204**, 727–731.
Chesher, G. B. & Thorp, R. H. (1965). *Br. J. Pharmac. Chemother.*, **25**, 288–294.
Edge, H. D. (1955). *J. Physiol., Lond.*, **127**, 54–68.
Emmelin, H. & Feldberg, W. (1947). *Ibid.*, **106**, 482–502.
Gaddum, J. H., Hameed, K. A., Hathaway, D. E. & Stephens, F. F. (1955). *Q. Jl exp. Physiol.*, **40**, 49–74.
Garry, R. C. (1928). *J. Physiol., Lond.*, **66**, 235–248.
Gillespie, J. S. & Wishart, M. (1957). *Ibid.*, **135**, 45P–46P.
Gross, L. & Clark, A. J. (1923). *Ibid.*, **57**, 457–460.
Gruber, C. M. (1933). *Physiol. Rev.*, **13**, 497–609.
Gyermek, L. (1961). *Am. J. Physiol.*, **201**, 325–328.
Henderson, V. E. & Roepke, M. H. (1934). *J. Pharmac. exp. Ther.*, **51**, 97–111.
Huković, S., Rand, M. J. & Vanov, S. (1965). *Br. J. Pharmac. Chemother.*, **24**, 178–188.
Innes, I. R., Kosterlitz, H. W. & Robertson, J. A. (1957). *J. Physiol., Lond.*, **137**, 396–409.
Langley, J. H. & Anderson, H. K. (1895). *Ibid.*, **19**, 71–139.
Prasad, B. N. (1935). *Ibid.*, **85**, 249–266.
Singh, I. (1964). *Br. J. Pharmac. Chemother.*, **22**, 403–407.
Ursillo, R. C. (1961). *J. Pharmac. exp. Ther.*, **131**, 231–236.
Ursillo, R. C. & Clark, B. B. (1956). *Ibid.*, **118**, 338–347.
Vogt, M. (1943). *J. Physiol., Lond.*, **102**, 170–179.
West, T. C., Hadden, G. & Farah, A. (1951). *Amer. J. Physiol.*, **164**, 565–572.