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Acetylcholine in extracts and perfusates of urinary bladder

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Tissue extracts of the urinary bladder of the rat, cat and guinea-pig contain an acetylcholine-like substance which is considered to be stored predominantly in post-ganglionic nerve fibres. An acetylcholine-like substance was liberated into the fluid bathing the isolated bladder of the guinea-pig and its output was increased 200-fold after transmural stimulation. The acetylcholine-like substance, both from bladder extracts (rat, cat and guinea-pig) and perfusates (rat), was found at the same Rf value as acetylcholine chloride when separated by paper chromatography in three solvent systems. It is concluded that the substance was acetylcholine.

THE response of the urinary bladder to stimulation of its parasympathetic nerves is not blocked by antimuscarinic drugs such as atropine. This phenomenon has been reported in a number of species *in vivo* (Langley & Anderson, 1895; Henderson & Roepke, 1934; Edge, 1955; Vanov, 1965), as well as with *in vitro* preparations (Ursillo & Clark, 1956; Huković, Rand & Vanov, 1965; Chesher & Thorp, 1965). Several hypotheses to explain this anomaly have been proposed, amongst them the suggestion that the transmission process may include a non-cholinergic as well as a cholinergic component.

The evidence for the involvement of a cholinergic mechanism at the neuromuscular synapse of the bladder is convincing. Although the response to stimulation of the parasympathetic nerves to the bladder is resistant to blockade by high concentrations of antimuscarinic drugs, some initial reduction of the amplitude of contraction can usually be produced. A study by Ursillo (1961) of this atropine-sensitive portion of the response to nerve stimulation showed it to have the same degree of sensitivity to atropine as was found in tissues in which the antimuscarinic drugs produce a full blockade. The response of the bladder to stimulation of its parasympathetic nerves is potentiated by physostigmine, and the potentiated portion of the response can be abolished by atropine (Chesher & Thorp, 1965). The response of the bladder to parasympathetic nerve stimulation is abolished by hemicholinium (Huković & others, 1965) or botulinum toxin (Carpenter, 1963) if nerve stimulation is prolonged.

Acetylcholine-like activity has been reported to occur in tissue extracts of the bladder of the dog (Chang & Gaddum, 1933), and of the rat (Huković & others, 1965). These extracts were found to stimulate the frog rectus abdominis muscle preparation (Chang & Gaddum, 1933) and the guinea-pig ileum, and to depress the blood pressure of the pithed rat (Huković & others, 1965). The activity of the bladder extracts on the frog rectus abdominis muscle was potentiated by physostigmine, and on the guinea-pig ileum it resisted block by methysergide or diphenhydramine and was abolished by atropine or hyoscine. The activity of extracts was destroyed by boiling in alkali but resisted boiling in acid solution (Huković & others, 1965).

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These properties suggest that the active substance in the extracts is a choline ester, though they do not identify it as acetylcholine. Indeed, Chang & Gaddum (1933) pointed out that, when tested on the frog rectus abdominis muscle, extracts of dog bladder produced effects which differed sufficiently in their time relations from those of acetylcholine to suggest that they may really be due to some other choline ester.

Acetylcholine-like activity has also been demonstrated in perfusates of urinary bladder. Henderson & Roepke (1934) perfused the dog bladder *in situ* and reported the appearance of an acetylcholine-like substance in the perfusate after stimulation of the perivascular nerves. However, they did not exclude the possibility that this substance could have been liberated from autonomic ganglia. Carpenter & Rand (1965) reported acetylcholine-like activity in the fluid bathing the isolated rat bladder when the bladder was at rest, and a significantly higher activity after nerve stimulation by co-axial electrodes. In neither of these studies was the active substance in the perfusates identified as acetylcholine.

This paper describes experiments which confirm that extracts of bladder tissue from cat, guinea-pig and rat, and the perfusate of the rat and the guinea-pig bladder, contain a substance with the properties of a choline ester. When separated by paper chromatography, using three solvent systems, the acetylcholine-like activity of the extracts and the perfusates, and of acetylcholine chloride were found in the eluates having corresponding Rf values.

Experimental

Methylene blue staining. The bladders, rapidly excised after the animal had been stunned and bled, were gently stretched on glass cannulae with the serous surface outermost and stained by immersion for $\frac{1}{2}$ to 1 hr in a hypotonic methylene blue solution at 37° (Hillarp, 1946). After fixation in a solution of molybdate (8% w/v) the tissue was dehydrated, cleared and examined as a whole-mount under the light microscope. Better resolution, especially at higher magnifications, could be obtained by reducing the thickness of the whole-mount by removing the mucosa after dehydration.

Tissue extracts for acetylcholine estimation. Extracts were made by the methods of Beani & Bianchi (1963), using 0·01M citrate-phosphate buffer, pH 4, or with ice-cold 10% trichloroacetic acid, as described by Hebb, Krnjevic & Silver (1964). Tissue blanks for the trichloroacetic acid extracts were prepared as described by Hebb & others (1964), and those for the citrate-phosphate buffer extracts were prepared by boiling the tissue in 0·1N sodium hydroxide for 10 min. After cooling and neutralizing these blanks with 0·1N hydrochloric acid, an equal volume of 0·02M citrate-phosphate buffer, pH 4 was added. All tissue blanks were tested on the guinea-pig ileum to ensure the absence of acetylcholine-like activity.

The activity of the extracts was measured on the guinea-pig ileum preparation and check assays of some of the samples were made on the heart of the mollusc *Tapes watlingi*. The guinea-pig ileum was bathed in Tyrode solution (Feldberg, 1951) containing diphenhydramine 2×10^{-8} g/ml, maintained at 32° and aerated with oxygen, 95% and carbon dioxide, 5%. The *Tapes* heart was set up as described by Ladd & Thorburn (1955) and bathed in filtered sea water without aeration (Carroll & Chesher, 1965).

The acetylcholine equivalent was determined by matching the responses to the extract (diluted 1 : 5 or 1 : 10) with those produced by acetylcholine in tissue blank. In each instance, concentrations of acetylcholine were selected to produce responses both greater and smaller than those produced by the extract.

Extracts and tissue blanks were stored at −4° for up to 3 weeks before estimation of activity or application to chromatography paper was made. Five samples of acetylcholine added to tissue blank and stored under these conditions for 6 weeks were satisfactorily recovered when tested on the guinea-pig ileum preparation (% recovery = 96% ± 7·3).

Paper chromatography of tissue extracts. Ascending chromatograms on methanol washed and dried Whatman No. 3 mm paper were run in three solvent systems: (i) propanol–0·1N acetic acid (3 : 1); (ii) butanol–acetic acid–water (60 : 15 : 25); (iii) butanol–propanol–water (4 : 2 : 1). To each paper was applied 0·1 to 0·5 ml of citrate–phosphate buffer tissue extract from rat, cat or guinea-pig bladder. As a control, an equal volume of tissue blank and tissue blank containing acetylcholine (0·1 μg) were applied to the paper. The various extracts were developed in each of the three solvent systems. The samples were applied as a streak approximately 2 cm long, perpendicular to the direction of solvent flow, using the Agla micro-syringe. Chromatograms were run until the solvent front had moved 23 to 26 cm from the starting line. After drying, the papers were cut horizontally into sections, each encompassing 0·1 Rf unit, and the sections eluted in absolute ethanol for at least 30 min. The ethanol was then evaporated under reduced pressure at 55–60°, and the residue dissolved in 0·8 ml Tyrode solution. The eluates were tested on the guinea-pig ileum preparation using acetylcholine in Tyrode solution as control. For the testing of eluates, diphenhydramine was omitted from the Tyrode solution bathing the guinea-pig ileum.

The release of acetylcholine from the bladder (in vitro). The guinea-pig bladder was set up for transmural stimulation as described by Chesher & Thorp (1965), and incubated for 70 min at 37° in 25 ml of Tyrode solution containing mipafox, 10^{-5} g/ml. After washing by several changes of the bath fluid and adjusting the volume of Tyrode solution in the bath until it just covered the tissue, the bladder was stimulated by square wave pulses of 20 V, 2 msec duration, at a frequency of 20/sec. Stimulation was for 2 min followed by a 3 min rest period before another 2 min period of stimulation. After three or five such periods of stimulation (corresponding to 6 or 10 min total), the bath fluid was collected, its volume measured and its activity assayed on the guinea-pig ileum against a

standard solution of acetylcholine in Tyrode solution. For the determination of the resting output of acetylcholine, the bath fluid was collected for assay after the bladder had remained in it without stimulation for periods of 25 or 36 min.

Two perfusates, each of 8 ml vol, one from a bladder stimulated for 6 min and the other from a bladder allowed to rest for 36 min, were freeze-dried and the residues extracted with ethanol as described above.

Paper chromatography of bladder perfusates. The rat bladder was used for this study and was set up for transmural stimulation, incubated in mipafox and stimulated for periods of 2 min as described for the guinea-pig bladder. Extracts for chromatography were prepared by freeze-drying pooled samples of perfusate derived from a total of 100 min of stimulation of two or three bladders. Each of these pooled samples totalled approximately 80 ml of perfusate. To separate the acetylcholine-like substance from the inorganic salts in this volume of physiological solution, the freeze-dried residues were extracted with absolute ethanol. After centrifugation at approximately 4,000 rpm for 2 to 3 min to separate the ethanol-insoluble inorganic salts, the ethanolic solution was evaporated to small volume under reduced pressure at 55 to 60° and applied to chromatography paper. The same paper, method of application and solvent systems as described for the chromatography of tissue extracts were used. As controls, an equal volume of Tyrode solution, with or without added acetylcholine was freeze-dried, extracted with ethanol and applied to the paper. The developed chromatograms were eluted in the manner described for the tissue extracts, and the eluates were tested on the guinea-pig ileum preparation.

The responses of the guinea-pig ileum and the *Tapes* heart were recorded by means of a Thorp & Wilson (1965) isotonic transducer connected to a potentiometric pen writing recorder.

Results

As the aim of the present study was to identify the transmitter substance released by the post-ganglionic parasympathetic fibres to the smooth muscle of the bladder, a ganglion cell-free preparation was necessary in order to exclude the pre-ganglionic nerve endings as a possible source of acetylcholine-like activity. Whilst there is physiological and pharmacological evidence for the presence of intramural ganglion cells in the bladder of the guinea-pig (Chesher & Thorp, 1965; Chesher & James, 1966) and the cat (Gyermek, 1961) bladders, it seems that the rat bladder is devoid of ganglion cells (Huković & others, 1965). Histological confirmation of these observations was made in the present study.

Examination of whole-mounts of the bladder for ganglion cells. Ganglion cells were found in only two of ten rat bladders examined, and in two preparations only five to ten ganglion cells could be seen. This was in marked contrast to the bladder of the guinea-pig or cat. In ten cat and ten guinea-pig bladders abundant ganglion cells were seen. Although distributed in greatest number around the entrance of the ureters, ganglion

cells were found throughout the bladder musculature. From a rough count, using a low power magnification (× 200), of ganglion cells in two guinea-pig bladders, it was estimated that if the empty bladder were divided into two equal sections by a transverse cut, the urethral section would contain approximately 75% of the ganglion cell population.

Acetylcholine chloride equivalent of bladder extracts. The acetylcholine chloride equivalent of extracts of the bladder of rat, cat and guinea-pig, as determined on the guinea-pig ileum and the *Tapes* heart are shown in Tables 1–3. Some of the extracts were assayed on the two different preparations on different days, and were stored at −4° in the interim.

TABLE 1. ACETYLCHOLINE-EQUIVALENT DETERMINATIONS OF EXTRACTS OF URINARY BLADDER OF THE GUINEA-PIG

Tissue	Wet wt. (mg)	*Extraction method	Acetylcholine-equivalent estimates (μg/g) determined on (a) guinea-pig ileum	(b) *Tapes* heart	Mean
Whole bladder	520	TCA	—	1·9	1·9
	650	,,	—	1·2	1·2
	417	C-P	2·2; 1·8	2·5	2·2
	493	,,	1·4; 1·2	1·8	1·5
	388	,,	1·8; 1·9	—	1·9
	779	,,	2·3; 2·9	—	2·6
	292	,,	4·5	—	4·5
	240	,,	2·6	—	2·6
	387	,,	1·9	—	1·9
	297	,,	2·9	—	2·9
	263	,,	3·8	—	3·8
Urethral sections, bladders A & B	200	TCA	5·0	7·5; 3·8	5·4
Fundus sections, bladders A & B	250	,,	3·1	3·5; 5·3	4·0
Urethral sections, bladders C & D	350	,,	1·8	3·4; 3·3	2·8
Fundus sections, bladders C & D	420	,,	2·7	3·9; 3·9	3·5
Urethral sections, bladders E & F	513	C-P	1·3	—	1·3
Fundus sections, bladders E & F	668	,,	0·9	—	0·9
Urethral sections, bladders G & H	439	,,	3·1	—	3·1
Fundus sections, bladders G & H	395	,,	2·8	—	2·8

* TCA = Trichloroacetic acid. C-P = Citrate-phosphate buffer.

TABLE 2. ACETYLCHOLINE-EQUIVALENT DETERMINATIONS OF EXTRACTS OF URINARY BLADDER OF THE CAT

Tissue	Wet wt. (mg)	*Extraction method	Acetylcholine-equivalent estimates (μg/g) determined on (a) guinea-pig ileum	(b) *Tapes* heart	Mean
Whole bladder	4,130	TCA	—	1·5	1·5
	4,540	,,	—	1·4	1·4
	3,710	,,	2·6	3·0	2·8
	4,030	,,	3·7	2·5	3·1
	3,190	,,	3·2	2·7	3·0
	2,610	,,	5·5	5·4	5·5
	871	C-P	2·2; 2·5	3·1	2·6
	960	,,	2·1; 2·3	—	2·2
	885	,,	2·1; 2·2	—	2·2
	845	,,	2·0; 2·5	—	2·3
Urethral sections, bladders A & B	719	C-P	2·3	—	2·3
Fundus sections, bladders A & B	684	,,	2·6	—	2·6
Urethral sections, bladders C & D	705	,,	4·0	—	4·0
Fundus sections, bladders C & D	725	,,	3·9	—	3·9

TCA = Trichloroacetic acid. C-P = Citrate-phosphate buffer.

TABLE 3. ACETYLCHOLINE-EQUIVALENT DETERMINATIONS OF EXTRACTS OF URINARY BLADDER OF THE RAT

Tissue	Wet wt. (mg)	*Extraction method	Acetylcholine-equivalent estimates (μg/g) determined on (a) guinea-pig ileum	(b) *Tapes* heart	Mean
Whole bladder	100	TCA	—	0·6	0·6
	100	,,	6·3	5·0	5·7
	85	C-P	2·1; 4·1	3·3	3·2
	128	,,	1·8	—	1·8
	69	,,	3·2; 5·7	5·3	4·7
	93	,,	3·6	—	3·6
	97	,,	4·1	—	4·1
	90	,,	5·1; 5·1	—	5·1
	125	,,	3·7; 3·7	—	3·7
	79	,,	3·0; 6·0	—	4·5
	102	,,	6·1	—	6·1
	100	,,	3·9	—	3·9
	110	,,	1·9	—	1·9
	106	,,	2·8; 2·5	—	2·7
	110	,,	1·5; 2·5	—	2·0
	93	,,	2·8; 3·1	—	3·0
	182	,,	1·6; 1·6	—	1·6
	143	,,	3·8; 2·9	—	3·4
	86	,,	4·8; 5·9	—	5·4
	187	,,	2·8	—	2·8
	135	,,	2·5	—	2·5

TCA = Trichloroacetic acid. C-P = Citrate-phosphate buffer.

The means of all determinations, both on the guinea-pig ileum and the *Tapes* heart were (± standard deviation): rat, 3·4 ± 1·5; cat, 2·8 ± 1·1; guinea-pig, 2·7 ± 1·2 μg/g.

It will be seen from Tables 1–3 that there was good agreement in the values obtained from the determinations on the guinea-pig ileum with those obtained on the *Tapes* heart. Furthermore, the activity of the extracts prepared by the two methods employed also showed good agreement.

Acetylcholine equivalent of "ganglion-rich" and "ganglion-poor" areas. The bladders of 8 guinea-pigs and 4 cats were grouped in pairs and each bladder divided transversely into fundic ("ganglion-poor") and urethral ("ganglion-rich") halves. The two urethral and the two fundic sections of each pair of bladders were combined and extracts made to compare their acetylcholine equivalents.

The results shown in Tables 1 and 2 indicate that there was no detectable difference in the acetylcholine equivalents of these two bladder sections. Within each pair of bladders there was good agreement in the estimates of each of the combined halves, and the mean value of all the urethral section extracts did not differ significantly from the mean value of all the fundic section extracts.

These results indicated that any acetylcholine-like substance contained within the pre-ganglionic nerve endings did not contribute significantly to the total acetylcholine measured.

Pharmacological properties of the tissue extracts. Suitable concentrations of the bladder extracts stimulated the guinea-pig ileum and inhibited the spontaneous beating of the *Tapes* heart. The contraction of the guinea-pig ileum was reduced or could be abolished completely by suitable concentrations of atropine. Equal responses to acetylcholine in

tissue blank and to the bladder extract were reduced to the same extent by atropine. The activity of the extracts was abolished when they were boiled at pH 9·5 to 10, or when incubated with guinea-pig plasma. However, activity was not reduced when the extracts were boiled at pH 4, or incubated in guinea-pig plasma containing physostigmine ($1{\cdot}6 \times 10^{-5}$ g/ml).

All of these properties suggest that the active substance is a choline ester. However, there were some qualitative differences between the contractions produced by the tissue extracts and those induced by acetylcholine in the tissue blank. The contraction induced by the extract was often of slower onset and took longer to reach a maximum. These differences were more apparent when the responses had been reduced by atropine (Fig. 1). The substance responsible for this difference

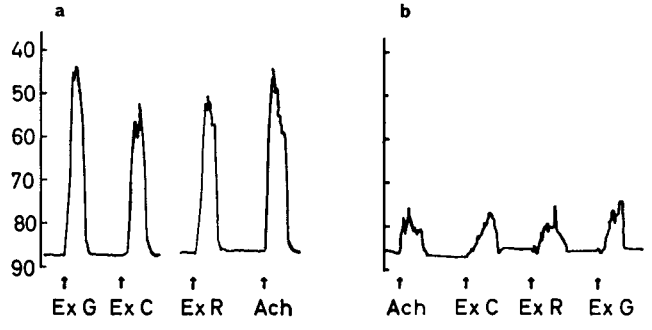

FIG. 1. Responses of the guinea-pig ileum, suspended in 2 ml Tyrode solution, to acetylcholine (Ach), $1{\cdot}25 \times 10^{-9}$ g/ml, and to tissue extracts of the bladder of the rat (Ex R), the guinea-pig (Ex G) and the cat (Ex C). In (a) normal Tyrode solution; in (b) the Tyrode solution contained atropine, 5×10^{-9} g/ml. Note the slower response to the tissue extract when compared with the response to acetylcholine. Divisions on ordinate = 0·5 inch. Magnification × 30.

is presumably destroyed by the procedure used in the preparation of the tissue blanks, because acetylcholine in the tissue blank did not behave in this manner.

Paper chromatography of bladder extracts. Only citrate-phosphate buffer extracts were used for chromatography, and to control the possible influence on the Rf values of other substances in the extracts each paper was spotted with tissue blank containing acetylcholine chloride, and with inactive tissue blank. Fifteen chromatograms were developed, eluted and tested on the guinea-pig ileum. In butanol–propanol–water the approximate Rf values of the active eluates were 0·1–0·2 for acetylcholine and also for the extract and perfusate. In butanol–acetic acid–water the Rf values were 0·45–0·55 for these eluates and in propanol–acetic acid they were 0·4–0·5. The results show that the activity determined on the guinea-pig ileum is clearly confined to eluates derived from paper equivalent to 0·1 Rf unit and in each of the three solvent systems used the active eluates of both the extract and of acetylcholine corresponded to the same Rf values. Furthermore there was no species difference, the bladder extracts from rat, cat and guinea-pig all behaving similarly. No activity was found in control eluates.

The qualitative differences seen in the responses of the guinea-pig ileum to acetylcholine and to the tissue extracts were not seen with the active chromatographic eluates of the extracts. The responses to the active eluates of both acetylcholine and extract were reduced by atropine to the same extent (Fig. 2).

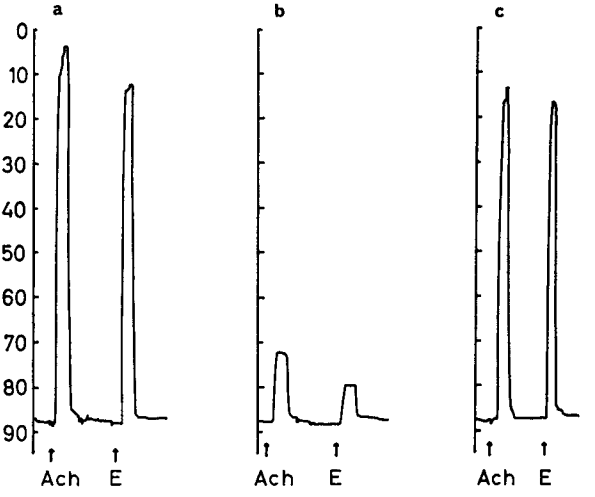

FIG. 2. Responses of the guinea-pig ileum to the active eluates from a chromatogram to acetylcholine (Ach) and a tissue extract of guinea-pig bladder (E). Eluates were obtained from paper strips of similar Rf value. In (a) normal Tyrode solution was used; in (b) and (c) the Tyrode solution contained atropine, $7{\cdot}5 \times 10^{-9}$ g/ml. In (c) the amounts of acetylcholine or tissue extract added to the guinea-pig ileum were increased tenfold. Divisions on ordinate = 0·5 inch. Magnification × 30.

The release of transmitter from the guinea-pig isolated bladder. Acetylcholine-like activity was detected in the perfusate of the resting guinea-pig bladder, and electrical stimulation of its intrinsic nerves increased the output of this substance approximately 200 times. The results of 16 determinations on six guinea-pig bladders are shown in Table 4.

TABLE 4. THE RELEASE OF ACETYLCHOLINE-LIKE SUBSTANCE INTO THE FLUID BATHING THE ISOLATED BLADDER OF THE GUINEA-PIG. DETERMINATIONS MADE ON THE GUINEA-PIG ILEUM

Resting output (ng/hr)	Stimulated output (ng/hr)
4*	400
5	600
8*	600
	1,300*
8*	2,000
8	2,400
10	2,500*
	3,000
10	3,000
Mean = $7{\cdot}6 \times 10^{-9}$ g/hr	Mean = $1{\cdot}8 \times 10^{-6}$ g/hr

* Freeze dried perfusates.

The response of the guinea-pig ileum to the perfusate, unlike that to tissue extract, was indistinguishable from the response to acetylcholine. Responses to acetylcholine and to the perfusate were reduced to the same extent by atropine (Fig. 3).

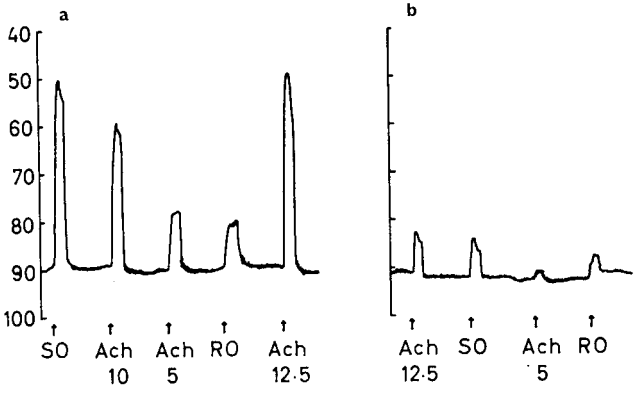

FIG. 3. (a) The responses of the guinea-pig ileum to perfusates from the guinea-pig bladder. Extracts of the freeze-dried residue of perfusates containing the transmitter output from 6 min transmural stimulation or of 36 min resting release were each dissolved in 1 ml of Tyrode solution. SO = 0·2 ml of a 1:10 dilution of the stimulated output extract. RO = 0·2 ml of the resting output extract. Ach = acetylcholine chloride, g/ml $\times 10^{-10}$. In (b) the Tyrode solution contained atropine, 5×10^{-10} g/ml. Divisions on ordinate = 0·5 inch. Magnification × 30.

On standing for 2–3 hr at room temperature all the perfusates lost most of their activity.

Paper chromatography of bladder perfusates. The rat bladder was used. The virtual absence of ganglion cells in this preparation excludes the pre-ganglionic nerve endings as a possible source of the acetylcholine-like activity.

As the preparation of the perfusates for chromatography involved freeze-drying, the effect of this treatment on the activity recovered was tested. The perfusates from two guinea-pig bladders were freeze-dried and the residues, extracted as described previously, were tested on the guinea-pig ileum. The results (Table 4, see *) were in good agreement with those obtained by the direct assay of perfusates. Furthermore, these experiments showed that the active substance, like acetylcholine, is soluble in absolute ethanol.

Three chromatograms were developed and the approximate Rf values of the acetylcholine-like substance in the perfusate were found to be the same as for acetylcholine chloride in each of the three solvent systems used. Control extracts of Tyrode solution were inactive. The response of the guinea-pig ileum to the active eluate of both the perfusate and the acetylcholine was reduced by atropine to the same extent. An increase in the concentration of both agonists overcame the blockade by atropine (Fig. 4).

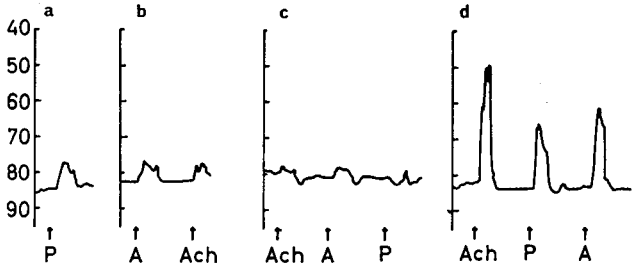

FIG. 4. After chromatography of a sample of perfusate from the rat bladder following transmural stimulation, and of acetylcholine added to perfusion fluid, eluates were made from strips of paper corresponding to the Rf value of acetylcholine. P = 0·1 ml of the eluate from the chromatogram of rat bladder perfusate. A = 0·1 ml of 1 : 10 dilution of the eluate from the chromatogram of acetylcholine in perfusion solution. Ach = acetylcholine, 5×10^{-10} g/ml. In (a) and (b) ileum was bathed in normal Tyrode solution; in (c) and (d) in the presence of atropine (5×10^{-10} g/ml). In (d) the amounts of A or Ach added to the ileum were increased tenfold, and of P were increased threefold. Divisions on ordinate = 0·5 inch. Magnification × 30.

Discussion

The results presented here provide evidence for the presence of acetylcholine-like activity in tissue extracts of the urinary bladder of the guinea-pig, the cat and the rat. The extracts, prepared by two procedures, had properties similar to those previously described by Chang & Gaddum (1933) for the dog bladder and by Huković & others (1965) for the rat bladder.

The differences in the time relations of the response of guinea-pig ileum to tissue extracts or to acetylcholine in the tissue blank appear to be similar to those reported by Chang & Gaddum (1933), who tested extracts of the dog bladder on the frog rectus abdominis muscle. This difference may be due to an interfering substance in the tissue extracts which reduced the rate of contraction of the guinea-pig ileum to acetylcholine. This effect was more apparent when the response of the guinea-pig ileum to acetylcholine had been reduced by atropine. This interfering substance was destroyed by the treatment used to prepare the tissue blanks, and was separated from the acetylcholine-like substance by paper chromatography. However, as it could not be detected in the perfusates of bladder after transmural stimulation, and its effect on the ileum was one of inhibition, this substance does not appear to be associated with the transmission of excitation at the neuromuscular synapse of the bladder.

The acetylcholine-like activity was found in approximately equivalent concentrations in the "ganglion rich" (urethral) and the "ganglion poor" (fundic) segments of the cat and guinea-pig bladder, and in the ganglion cell-free rat bladder. Therefore, assuming that the ester is found predominantly in nervous tissue, the concentrations reported represent ester stored in the post-ganglionic fibres.

The estimate of the acetylcholine-equivalent of the active substance found in the perfusate from the guinea-pig bladder, both at rest and after nervous stimulation, were in agreement with that reported for the rat bladder by Carpenter & Rand (1965). The participation of this substance in the neuromuscular transmission of the bladder is suggested by the 200-fold increase in output following nervous stimulation. Furthermore, Carpenter & Rand (1965) have shown that the transmural stimulation of the rat bladder does not release the acetylcholine-like substance if the post-ganglionic nerve fibres have degenerated.

After separation by paper chromatography, the acetylcholine-like substance of the rat, cat and guinea-pig bladder extracts and of the perfusates collected after transmural stimulation of the rat bladder was found in the eluates corresponding to the same Rf value as those containing acetylcholine chloride. The solvent systems chosen have been shown to provide a satisfactory separation of the choline esters (Whittaker, 1963; Cobbin, Leeder & Pollard, 1965).

It is concluded that the chromatographic and pharmacological properties of the acetylcholine-like substance in extracts and perfusates of the bladder are compatible with it being acetylcholine.

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