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The actions of some cholinomimetic drugs on the isolated taenia of the guinea-pig caecum

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1. The actions on the taenia of 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343), N-benzyl-3-pyrrolidyl acetate methobromide (AHR-602), tetramethylammonium (TMA) and choline phenyl ether have been examined and compared with the actions of acetylcholine, nicotine and 1,1-dimethyl-4-phenylpiperazinium (DMPP).

2. Responses of the taenia to these agonists are quantitatively and often qualitatively dependent on the tone of the preparation. A method is described which makes allowance for the effect of tone on heights of contractions.

3. Acetylcholine, McN-A-343 and AHR-602 produced only contractions; TMA produced contractions or biphasic responses; and choline phenyl ether, nicotine and DMPP produced contractions, relaxations or biphasic responses.

4. The mode of action of these compounds has been analysed by means of hyoscine, ganglion-blocking drugs, tetrodotoxin and local anaesthetics.

5. It is concluded that acetylcholine, McN-A-343, AHR-602, TMA and choline phenyl ether act on muscarinic receptors in the smooth muscle. Choline phenyl ether has an additional action on nicotinic receptors of cholinergic neurones. Nicotine and DMPP act on nicotinic receptors of cholinergic neurones and of inhibitory neurones. An action on the latter is sometimes also seen with TMA and choline phenyl ether.

6. With nicotine or DMPP, and TMA or choline phenyl ether in the presence of hyoscine, part of the contraction phase of biphasic responses (in which a contraction follows relaxation) is best explained as being triggered by the initial relaxation—that is, as a "rebound contraction".

7. None of the compounds tested appeared to exert an atropine-sensitive action on neurones.

8. In the presence of hyoscine high concentrations of agonists can act on sites not involved with lower concentrations.

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9. The degree of antagonism obtained with procaine against compounds acting on muscarinic receptors on the smooth muscle varies with the compound tested.

The effects on the isolated taenia of the guinea-pig caecum of the ganglion stimulants nicotine and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) vary with the tone of the preparation; accordingly, contractions, relaxations or complex responses consisting of both effects have been obtained with these substances (Weis, 1962; Burnstock, Campbell & Rand, 1966; Akubue, 1966a). The effects on the same preparation of other nicotine-like ganglion stimulants, such as choline phenyl ether and tetramethylammonium (TMA), and the effects of muscarinic ganglion stimulants, such as 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) and N-benzyl-3-pyrrolidyl acetate methobromide (AHR-602), have not been extensively investigated.

In this paper the modes of action of McN-A-343, AHR-602, TMA and choline phenyl ether on the isolated taenia of the guinea-pig caecum have been investigated in detail and compared with those of acetylcholine, nicotine and DMPP.

Methods

Adult guinea-pigs of either sex, weighing 300–800 g, were killed by a blow on the head and a single taenia was dissected from the caecum. Preparations consisting of a length of 2–3 cm (unstretched) were suspended in McEwen's (1956) solution in a thermostatically controlled organ bath (10 ml. capacity) at 37° C. The bath fluid was gassed with 95% oxygen and 5% carbon dioxide. Responses were recorded on a smoked drum with an isotonic frontal writing lever producing an approximately six-fold magnification and exerting a tension of 1 g. The McEwen's solution had the following composition (expressed as g/l. of distilled water): NaCl, 7·6; KCl, 0·42; $CaCl_2$, 0·24; $NaHCO_3$, 2·1; NaH_2PO_4, 0·143; glucose, 2·0; sucrose, 4·5.

Agonists were added in small volumes (0·1–0·5 ml.) to the organ bath with a syringe. Periods of contact with the tissue varied, and are stated in the text. The tissue was then washed by overflow for 10 sec, during which time 40–50 ml. of prewarmed McEwen's solution passed through the organ bath. In the records illustrated in the figures, the kymograph was not stopped during washing. The interval between testing agonists was 4 min or more. Other drugs were added to the organ bath either immediately after a washout, or 2–6 min before an agonist, or they were added to the McEwen's solution in the reservoir.

Agonists used were acetylcholine chloride, TMA chloride, AHR-602, McN-A-343, choline phenyl ether bromide, DMPP and nicotine hydrogen tartrate.

Substances used for analysis of the mode of action of agonists were cinchocaine hydrochloride, cocaine hydrochloride, hexamethonium bromide, hyoscine hydrobromide, mecamylamine hydrochloride, nicotine hydrogen tartrate, pempidine hydrogen tartrate, procaine hydrochloride, tetrodotoxin, trimetaphan camphorsulphonate, and (+)-tubocurarine chloride.

All concentrations given in the text refer to the concentration of the above compounds in the organ bath.

Results

Spontaneous activity, tone and effects of tone on responses of the isolated taenia to agonists

The taenia had spontaneous activity which varied from one preparation to another and was roughly proportional to its tone. It was convenient to define the tone of individual preparations as low, medium or high according to the magnitude of responses obtained with acetylcholine (Fig. 1). Burnstock *et al.* (1966) used a similar procedure but based their assessment of tone on responses to catecholamines.

Preparations with a low tone usually had considerable spontaneous activity consisting of rapid, regular pendular movements of large amplitude. In a few preparations with a low tone, the spontaneous activity was slight, absent or alternated between marked and slight activity. Preparations with a low tone had the highest sensitivity to acetylcholine.

Preparations with a medium tone had irregular and relatively slow spontaneous movements often with transient phases of faster spontaneous activity. During the course of experiments the tone of such preparations frequently fell and when this occurred the amplitude, regularity and frequency of spontaneous movements increased. In preparations with a medium tone the threshold concentration for acetylcholine was usually 0·003–0·01 μg/ml.

Preparations with a high tone exhibited little or no spontaneous activity and had the lowest sensitivity to acetylcholine. They too, often showed a decline in tone throughout an experiment.

To study the relationship between the tone of a preparation and the height of contraction obtained with individual concentrations of acetylcholine, several experiments were carried out in which different concentrations of acetylcholine were tested repeatedly. It was found that in any given experiment the slope of the concentration (log) : response curve was inversely related to tone and that within the range

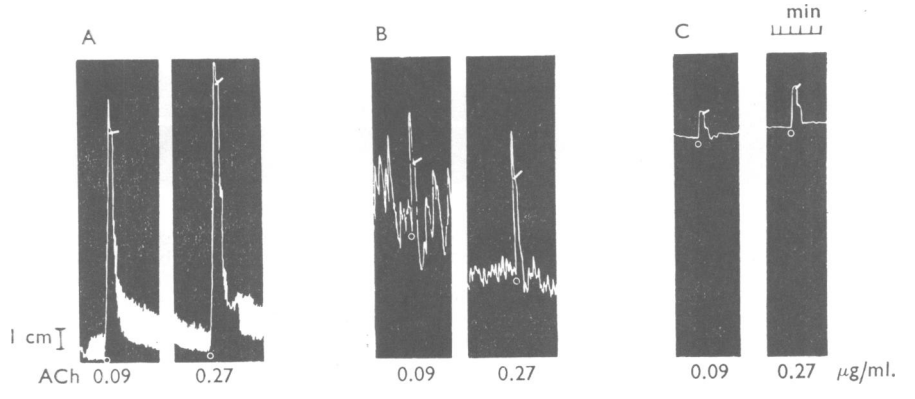

FIG. 1. Relationship between tone and response to acetylcholine in three different preparations, with low (A), medium (B) and high (C) tone. At ○, acetylcholine (ACh) was added to the organ bath in the final concentrations stated and oblique white marks indicate washouts. Time marker: 1 min ; vertical calibration: 1 cm on kymograph tracing.

of observed changes in tone, responses obtained with a given concentration of acetylcholine were best expressed as

$$Y_B = Y_A + kd$$

where Y_B and Y_A are heights of responses obtained with a given concentration of acetylcholine at a lower and a higher level of tone respectively, k is a constant and called the correction factor, and d represents the difference in the level of tone expressed as cm difference between the positions of the writing point at the times of addition of acetylcholine to the organ bath.

Figure 2 illustrates the calculation of k from results of an experiment in which four concentrations of acetylcholine were used on two occasions. It also shows that k increases as the concentration of acetylcholine is increased. The use of the expression $Y_B = Y_A + kd$ is justified by the finding that the k values for a given concentration of acetylcholine were fairly constant for different experiments.

The same relationship between tone and response applied to experiments with other agonists and Table 1 shows mean values for k derived from experiments with acetylcholine and TMA.

Using the expression $Y_{corr} = Y_{obs} + kd'$ and the mean values for k, all the heights of observed responses (Y_{obs}) recorded in an individual experiment were converted into heights (Y_{corr}) which would have been obtained had the tone of the preparation remained constant throughout the experiment at the lowest level at which tests were carried out ; d' is the difference between this tone and that preceding the addition

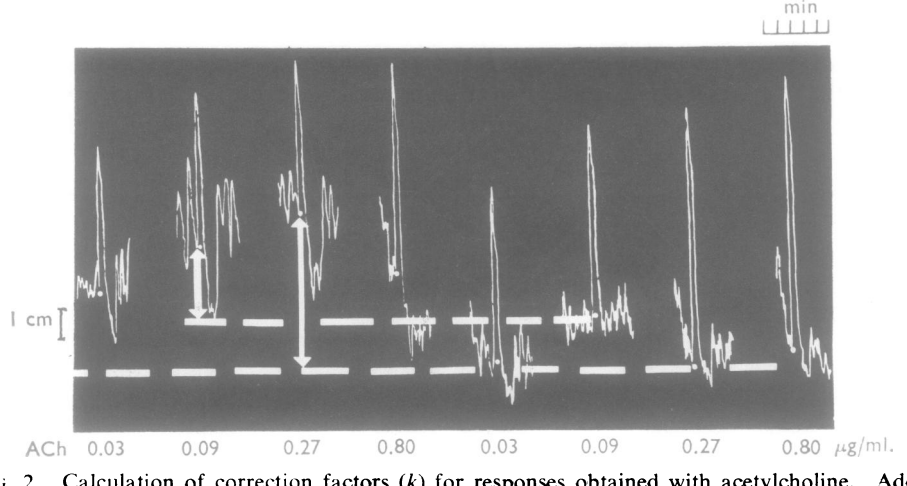

FIG. 2. Calculation of correction factors (k) for responses obtained with acetylcholine. Additions of acetylcholine (ACh), in the final concentrations stated, are marked by white dots. The value of k for any one concentration of acetylcholine is obtained from the equation $k = (Y_B - Y_A)/d$. The smaller of the two double-headed arrows represents d for the two tests with acetylcholine 0·09 µg/ml. and the larger double-headed arrow represents d for the two tests with acetylcholine 0·27 µg/ml. Time marker: 1 min ; vertical calibration: 1 cm on kymograph tracing. Evaluation of k from the results presented in the figure is as follows:

Concentration of acetylcholine (µg/ml)	0·03	0·09	0·27	0·80
Height of response at high tone (Y_A cm)	4·3	4·7	4·65	6·0
Height of response at low tone (Y_B cm)	5·4	5·75	7·8	8·1
Difference in tone (d cm)	2·4	1·9	4·55	2·6
Correction factor (k)	0·46	0·55	0·69	0·81

of agonist at a higher tone. Table 2 illustrates this method for obtaining corrected responses from the record presented as Fig. 2.

Only results which are corrected for variations of responses arising from changes in tone of a preparation give information which can be used for comparisons between the actions of agonists, assessment of concentration : response curves and interpretation in studies designed to locate the mode of action of agonists. Information concerning these aspects and presented in the following text, therefore, is based either on corrected responses whenever changes in tone did occur between tests, or on results obtained in experiments in which no changes in tone occurred.

Actions of agonists

Acetylcholine

This substance always produced a contraction, even in very high concentrations (30 μg/ml.). The usual response of the taenia to acetylcholine (0·001–30 μg/ml.) was a rapid contraction which quickly reached a maximum, but was not fully maintained during the 30 sec the agonist remained in contact with the tissue. In some preparations with a high tone, however, the contraction developed more slowly, only reaching a maximum after 30 sec or even later.

McN-A-343 and AHR-602

These substances, in concentrations of 0·01–50 μg/ml. and 1–81 μg/ml. respectively, produced contractions which developed more slowly than those produced by

TABLE 1. *Mean correction factors (k) for various concentrations of acetylcholine and tetramethylammonium*

Acetylcholine		Tetramethylammonium	
Concentration (μg/ml.)	Correction factor mean±S.E.	Concentration (μg/ml.)	Correction factor mean±S.E.
0·01	0·260±0·045 (27)	1	0·254±0·067 (7)
0·03	0·403±0·038 (27)	3	0·317±0·048 (10)
0·09	0·636±0·049 (27)	6	0·536±0·049 (4)
0·27	0·715±0·051 (27)	9	0·808±0·061 (10)
0·80	0·750±0·049 (27)	27	0·966±0·073 (9)
		81	1·095±0·072 (10)

Figures in parentheses indicate numbers of experiments.

TABLE 2. *Method adopted for correcting observed responses for variations in responses arising from differences of tone using data drawn from Fig. 2.*

Concentration of acetylcholine (μg/ml.)	0·03		0·09		0·27		0·80	
Observed response (cm) (Y_{obs})	4·30	5·40	4·70	5·75	4·65	7·80	6·0	8·10
Tone level (cm) (d)	2·45	0·05	3·55	1·65	4·55	0	3·15	0·55
Correction factor (Mean value from Table 1) (k)	0·403		0·636		0·715		0·75	
Corrected response (cm) (Y_{corr})	5·29	5·42	6·96	6·80	7·90	7·80	8·36	8·51

Corrected responses (Y_{corr}) are obtained from the observed response (Y_{obs}) plus correction factor (k) times the difference in tone level (in cm) between the position of the writing point when acetylcholine was added and the lowest position of the writing point preceding any addition of acetylcholine (d'). The latter is marked by the lower of the two broken lines in Fig. 2.

acetylcholine, usually taking up to 60 sec or more to reach a maximum. The concentration : response curves for these agonists were steeper than that for acetylcholine and threshold concentrations in preparations with a medium tone were 0·3–1 μg/ml. for McN-A-343 and 1–3 μg/ml. for AHR-602. Relaxations were never observed with these agonists even in preparations with a high tone.

Tetramethylammonium

In concentrations of 0·1–81 μg/ml., TMA usually produced a contraction. However, the contraction was sometimes preceded by a relaxation when high concentrations (10–81 μg/ml.) were tested on preparations with a high tone. The contraction, or the contraction phase of a biphasic response, usually required 60 sec or more to reach a maximum. The threshold concentration was generally 1–3 μg/ml. in preparations with a medium tone and the slope of the concentration : response curve was steeper than that for acetylcholine.

Choline phenyl ether

The range of concentrations used was 0·1–81 μg/ml. In preparations with a low tone, the substance produced only a contraction which took 60 sec or more to reach a maximum. Sometimes the response consisted of an initial large rapid contraction interrupted by or followed by a momentary decrease in tone before the sustained response became manifest. When the tone was medium, low concentrations (1–9 μg/ml.) produced a contraction only, but higher concentrations caused a biphasic response both components of which were concentration dependent. The contraction phase of a biphasic response differed from that produced by nicotine in that it was fully maintained over 60 sec. In a few experiments a three-phase response was seen as with nicotine. On preparations with a high tone, concentrations of 9–81 μg/ml. produced relaxation only.

Nicotine

In preparations with a low tone, nicotine (0·3–81 μg/ml.), produced a contraction which was not fully maintained during either 30 or 60 sec contact times. Occasionally there was an initial small contraction which was momentarily interrupted by a pause or even by a slight decrease in tone before the contraction continued to its maximum. Relaxations below the base line were never observed. In preparations with a medium tone the nature of the response depended on the concentration of nicotine. In general, lower concentrations (1–9 μg/ml.) produced a contraction, but higher concentrations caused a biphasic response consisting of a rapid relaxation followed by a contraction which was not fully maintained during the period of contact between the agonist and the tissue. Occasionally a three-phase response occurred which consisted of a small transient contraction, then a relaxation, followed in turn by a marked contraction. In preparations with a high tone, nicotine (9–81 μg/ml.) produced only a relaxation and the preparation often began to recover from the relaxation while the agonist remained in contact with the tissue. If the tone changed spontaneously during an experiment, the response to nicotine changed accordingly. In all experiments high concentrations of nicotine (81 μg/ml.) produced desensitization to nicotine, and prolonged washing was necessary to restore the initial sensitivity.

DMPP

In concentrations of 0·1–81 μg/ml., this substance had essentially similar actions to nicotine and both substances were approximately equiactive on a molar basis. In preparations with a medium tone, however, a biphasic response was more frequently produced with all concentrations of DMPP. Nevertheless, as with nicotine, the contraction was the predominant phase with lower concentrations of DMPP.

Studies concerning the site of action of agonists

In the experiments described below, the effects of hyoscine, ganglion-blocking drugs and local anaesthetics on the actions of the agonists were investigated. For each agonist a contact time was chosen which, as far as possible, allowed the main features of the response to develop fully. Generally, the contact time for acetylcholine was 30 sec and for other agonists 60 sec.

Effect of hyoscine on actions of agonists

Hyoscine (0·1–1 μg/ml.) usually did not affect the tone, but in a few experiments it caused a slight decrease.

Hyoscine (0·1–1 μg/ml.) antagonized contractions produced by acetylcholine (0·001–1 μg/ml.). In the presence of hyoscine higher concentrations of acetylcholine (10–100 μg/ml.) caused a relaxation or a biphasic response.

Contractions produced by AHR-602 and McN-A-343 were also antagonized by hyoscine (0·1–1 μg/ml.) and usually no relaxation was revealed, even when concentrations of up to 81 μg/ml. (AHR-602) or 50 μg/ml. (McN-A-343) were used. In two out of ten experiments, however, high concentrations of McN-A-343 (9–27 μg/ml.) produced a slow relaxation in the presence of hyoscine.

Contractions produced by low concentrations of TMA were antagonized by hyoscine (0·1–1 μg/ml.). In the presence of hyoscine higher concentrations of TMA (10–81 μg/ml.) produced a relaxation or a biphasic response. The contraction phase of the biphasic response was always smaller than the contraction produced by the same concentration of TMA in the absence of hyoscine.

Hyoscine antagonized the contraction produced by choline phenyl ether ; moreover, if the concentration of agonist was 3 μg/ml. or higher, a relaxation or a biphasic response appeared. Hyoscine altered an initially biphasic response to choline phenyl ether by increasing the relaxation and decreasing the contraction.

Hyoscine antagonized the contraction produced by nicotine or DMPP ; again, if the concentration of these agonists was of the order of 5–10 μg/ml. or higher, the responses were converted to a relaxation or a biphasic response. The contraction phase of a biphasic response produced by nicotine or DMPP in the presence of hyoscine was often as large as, or occasionally even larger than, the contraction produced by the agonists in the absence of hyoscine (Fig. 3). When the response to nicotine or DMPP was biphasic in the absence of hyoscine the relaxation was increased in the presence of hyoscine and the contraction phase either reduced or unaltered.

Effect of ganglion-blocking drugs on actions of agonists

Hexamethonium

Hexamethonium (5–30 μg/ml.) was without effect on the tone of the preparation but in a concentration of 300 μg/ml. increased it.

Hexamethonium (5–300 μg/ml.) had very little effect on contractions produced by acetylcholine, McN-A-343 or AHR-602 if comparisons were based on corrected responses (Figs. 4 and 5). Contractions, or the contraction phase of biphasic responses produced by TMA (Fig. 5) or choline phenyl ether were also usually unaffected or only slightly reduced by hexamethonium (5–100 μg/ml.) as assessed from corrected responses. In preparations with a low tone a definite reduction of the initial phase of contraction produced by choline phenyl ether was obtained with hexamethonium as shown in Fig. 4. Contractions produced by nicotine or DMPP (Fig. 4) were markedly reduced or abolished by hexamethonium (5–100 μg/ml.).

The relaxation phase of biphasic responses produced by TMA or choline phenyl ether was abolished by hexamethonium (5–10 μg/ml.). Biphasic responses and relaxations produced by nicotine or DMPP were also abolished or markedly reduced by hexamethonium (5–100 μg/ml.). High concentrations (15–81 μg/ml.) of these agonists in the presence of hexamethonium produced a small, slow contraction which was not antagonized by hyoscine.

Other ganglion-blocking drugs and (+)-tubocurarine

Mecamylamine (5–10 μg/ml.), nicotine (15 μg/ml.), trimetaphan (5 μg/ml.), pempidine (3–60 μg/ml.) and (+)-tubocurarine (5 μg/ml.) had essentially similar

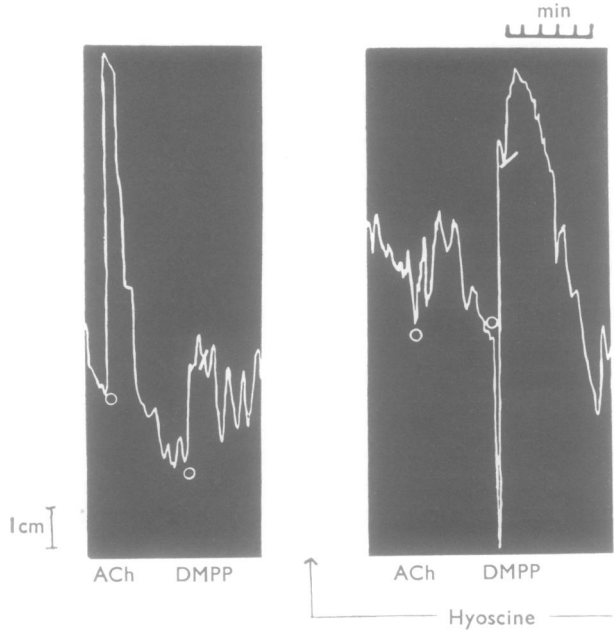

FIG. 3. Effect of hyoscine 1 μg/ml. on responses to acetylcholine (ACh) 0·015 μg/ml. and DMPP 15 μg/ml. Addition of agonists is marked by ○; the oblique white lines indicate points at which DMPP was washed out. Time marker: 1 min; vertical calibration: 1 cm on kymograph tracing.

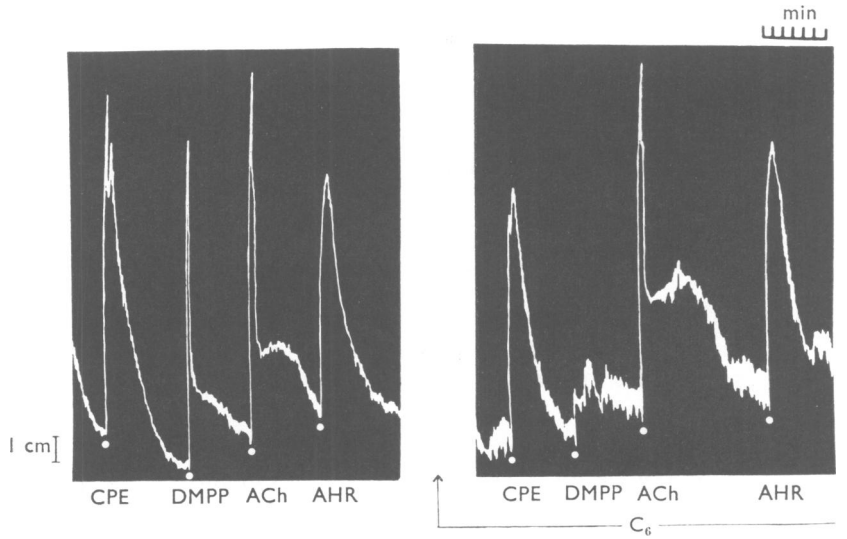

FIG. 4. Effect of hexamethonium (C_6) 5 μg/ml. on contractions produced by choline phenyl ether (CPE) 5 μg/ml., DMPP 3 μg/ml., acetylcholine (ACh) 0·3 μg/ml. and AHR-602 (AHR) 4 μg/ml. added at the white dots. Note that only the initial part of the response to choline phenyl ether was reduced by hexamethonium. Time marker: 1 min; vertical calibration: 1 cm on kymograph tracing.

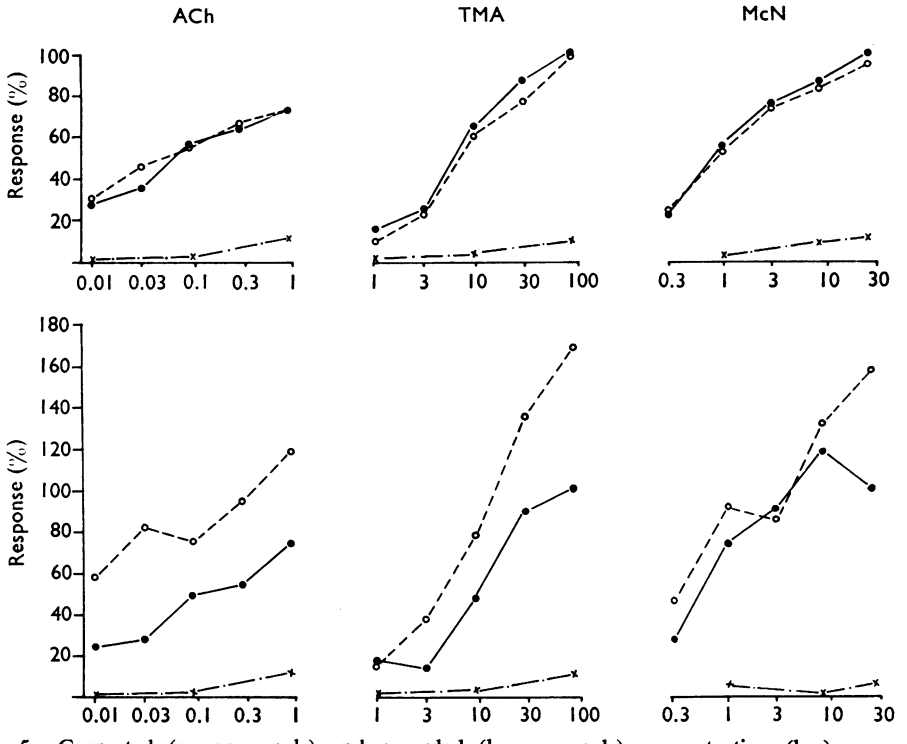

FIG. 5. Corrected (upper panels) and recorded (lower panels) concentration (log): response curves for acetylcholine (ACh), tetramethylammonium (TMA) and McN-A-343 (McN) in the absence (●——●) and presence (○- - -○) of hexamethonium (100 μg/ml.) and hexamethonium (100 μg/ml.) plus hyoscine (0·1 μg/ml.) (×-·-·-×). Responses to TMA and McN-A-343 are expressed as a percentage of the response obtained with the highest concentration of these agonists used in the absence of any antagonist. Responses to acetylcholine were obtained on the same preparations as those for TMA and are expressed as a percentage of the response to the highest concentration of TMA. Each point represents the mean of three experiments.

effects to hexamethonium on responses produced by agonists. The effects of pempidine and (+)-tubocurarine on the relaxation produced by choline phenyl ether in preparations with a high tone was also investigated. In such preparations, both drugs changed the response to choline phenyl ether to a contraction.

Effect of tetrodotoxin on actions of agonists

Tetrodotoxin (0·1 μg/ml.) caused a slight increase in tone of the taenia in about half the experiments but in the others it did not affect it. In some experiments the amplitude of spontaneous movements was reduced and sometimes this was accompanied by an increase in their frequency.

Contractions produced by acetylcholine, McN-A-343, AHR-602 or TMA were not affected by tetrodotoxin (0·1 μg/ml.), as assessed from corrected responses (Fig. 6). Contractions, or the contraction phase of biphasic responses produced by choline phenyl ether were also unaffected by tetrodotoxin (0·1 μg/ml.), whereas the relaxation phase of a biphasic response obtained with this agonist was abolished. In preparations with a high tone, in which high concentrations of choline phenyl ether caused only relaxation, the relaxation was absent after tetrodotoxin (0·1 μg/ml.) and the response to choline phenyl ether in concentrations of 3 81 μg/ml. was a

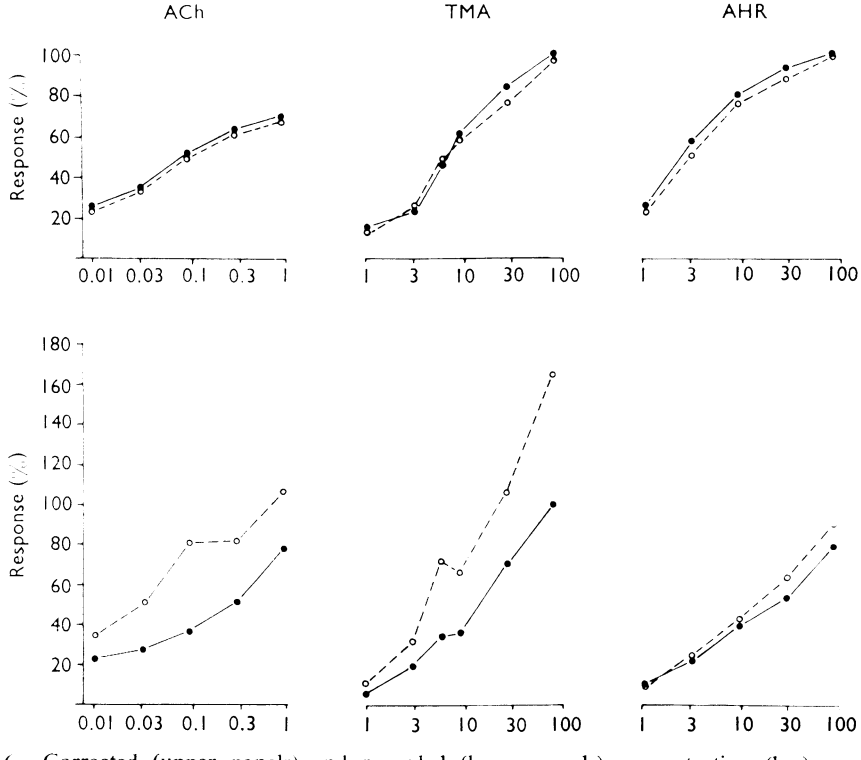

FIG. 6. Corrected (upper panels) and recorded (lower panels) concentration (log) : response curves for acetylcholine (ACh), tetramethylammonium (TMA), and AHR-602 (AHR) in the absence (●——●) and presence (○- - - -○) of tetrodotoxin (0·1 μg/ml.). Other details as in Fig. 5. Each point represents the mean of three experiments.

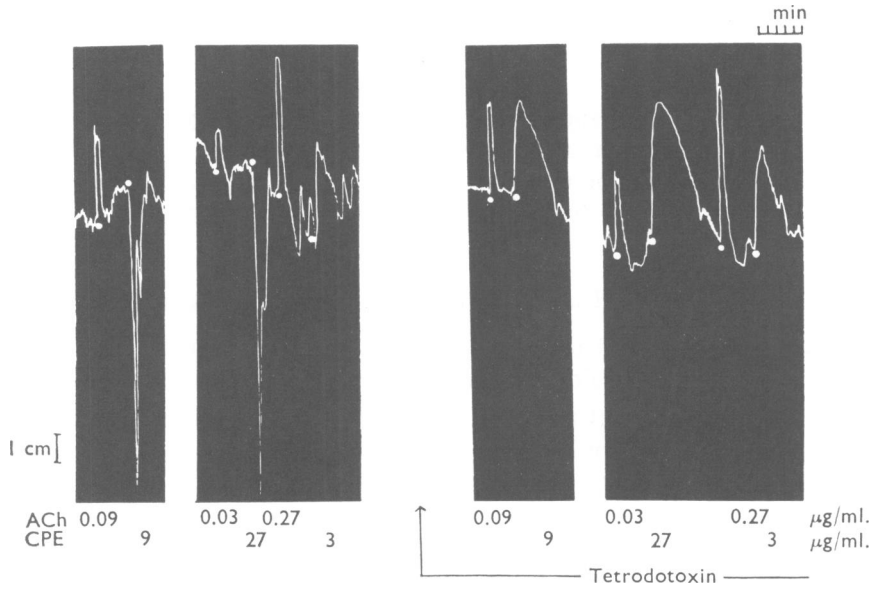

FIG. 7. Effect of tetrodotoxin 0·1 μg/ml. on responses to acetylcholine (ACh) and choline phenyl ether (CPE) added at the white dots. Time marker: 1 min ; vertical calibration: 1 cm on kymograph tracing.

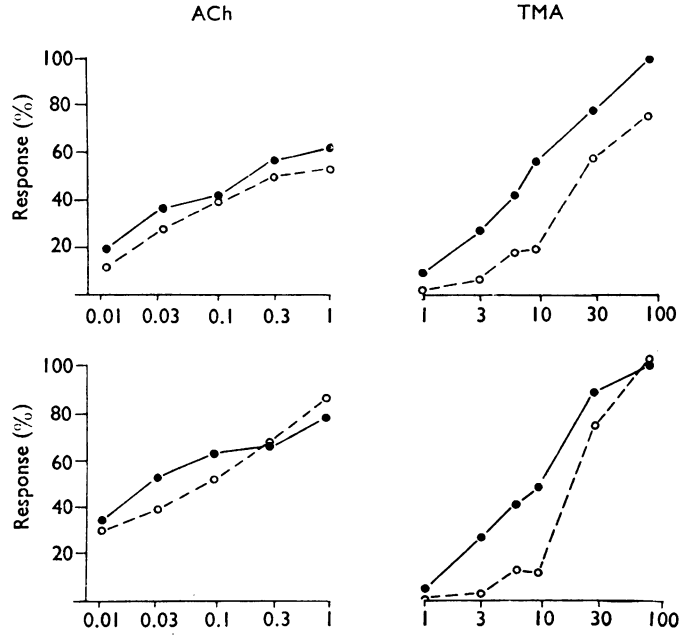

FIG. 8. Corrected (upper panel) and recorded (lower panel) concentrations (log) : response curves for acetylcholine (ACh) and tetramethylammonium (TMA) in the absence (●——●) and presence (○- - -○) of procaine (10 μg/ml.). Each point represents the mean of three experiments. Other details as in Fig. 5.

contraction (Fig. 7). These results with choline phenyl ether are contrary to those of Gershon (1966), who reported that tetrodotoxin abolished the response of the taenia to concentrations of the agonist below 10 μg/ml.

Contractions, biphasic responses or relaxations produced by nicotine or DMPP were markedly reduced or abolished by tetrodotoxin (0·01–0·1 μg/ml.). Sometimes a small, slow contraction or relaxation remained ; this is in contrast to the rapid response obtained with nicotine or DMPP before tetrodotoxin.

Effect of local anaesthetic drugs on actions of agonists

Cinchocaine, cocaine and procaine usually had no effect on the tone but sometimes cinchocaine and procaine increased it.

Cinchocaine (1–3 μg/ml.) and cocaine (10–50 μg/ml.) had the same effects as tetrodotoxin on the action of agonists. Higher concentrations of cinchocaine (5–10 μg/ml.) also reduced contractions produced by acetylcholine as well as by other agonists.

Procaine (10 μg/ml.) usually slightly reduced contractions produced by acetylcholine, but contractions produced by McN-A-343, AHR-602, TMA and choline phenyl ether were reduced to a greater extent as assessed from corrected responses. Figure 8 shows graphically the effects of procaine on responses to acetylcholine and TMA. Contractions produced by nicotine and DMPP were reduced by procaine to the greatest extent, being abolished in some experiments. The contraction phase of a biphasic response to choline phenyl ether, nicotine or DMPP was also markedly reduced or abolished by procaine.

Relaxations or the relaxation phase of biphasic responses produced by nicotine, DMPP or choline phenyl ether were also markedly reduced by procaine.

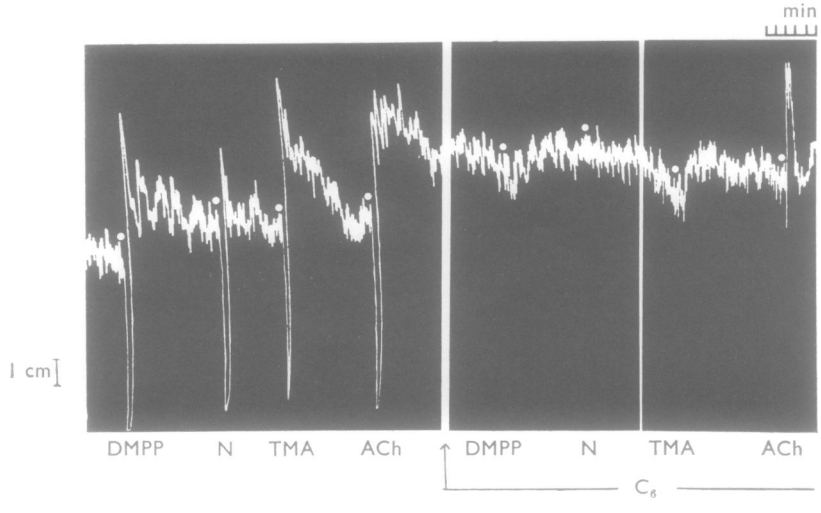

FIG. 9. Effect of hexamethonium (C_6) 5 μg/ml. on responses produced by DMPP 2 μg/ml., nicotine (N) 3 μg/ml., tetramethylammonium (TMA) 15 μg/ml. and acetylcholine (ACh) 15 μg/ml., added at the white dots in the presence of hyoscine (0·1 μg/ml.). Time marker: 1 min ; vertical calibration: 1 cm on kymograph tracing.

Effect of hexamethonium, tetrodotoxin and procaine on responses in the presence of hyoscine

Hexamethonium

In concentrations of 5 to 10 μg/ml. hexamethonium antagonized the relaxation and contraction phase of a biphasic response produced in the presence of hyoscine (0·1–1 μg/ml.) by TMA, choline phenyl ether, nicotine or DMPP (Fig. 9).

Usually the contraction phase of a biphasic response to acetylcholine in the presence of hyoscine (0·1–1 μg/ml.) was not reduced by hexamethonium although the relaxation was regularly markedly reduced or abolished. However, in some experiments in the presence of 1 μg/ml. of hyoscine both phases of the response were antagonized by hexamethonium. The contraction produced by acetylcholine in the presence of hyoscine 0·1 μg/ml. plus hexamethonium 5–10 μg/ml. was not affected by raising the concentration of hexamethonium to 100 μg/ml. but was reduced if the concentration of hyoscine was increased to 1 μg/ml.

Tetrodotoxin

In the presence of hyoscine (1 μg/ml.) tetrodotoxin (0·01–0·1 μg/ml.) changed the response produced by acetylcholine from a relaxation to a contraction.

In one experiment, the slow relaxation produced by McN-A-343 in the presence of hyoscine was reduced but not abolished, by tetrodotoxin (0·1 μg/ml.).

Relaxations produced in the presence of hyoscine (1 μg/ml.) by TMA, choline phenyl ether, nicotine or DMPP were antagonized by tetrodotoxin (0·01–0·1 μg/ml.) but a small slow relaxation sometimes remained as the response to these agonists.

Procaine

In the presence of hyoscine (1 μg/ml.) procaine (10–30 μg/ml.) had similar effects to hexamethonium or tetrodotoxin in reducing or abolishing relaxations or biphasic responses produced by acetylcholine, TMA, choline phenyl ether, nicotine or DMPP.

Discussion

Responses of the taenia to agonists are qualitatively and quantitatively dependent on the tone of preparations. Therefore, unless responses are corrected as described in the text, erroneous conclusions might be reached about the relative potency of agonists and their sites of action.

The contraction of the taenia produced by acetylcholine represents a muscarinic effect on the smooth muscle, because it was abolished by hyoscine and not affected by ganglion-blocking drugs, tetrodotoxin or local anaesthetics. A similar conclusion has been reached by Bülbring (1954, 1955), Burnstock *et al.* (1966) and Akubue (1966a). In the presence of hyoscine, high concentrations of acetylcholine caused relaxations or biphasic responses. The relaxation was antagonized by ganglion-blocking drugs, tetrodotoxin or local anaesthetics. This second action was therefore probably on nicotinic receptors of inhibitory neurones. Activation by nicotinic drugs of intramural inhibitory neurones in the intestine was detected after cholinergic paralysis with botulinum toxin or atropine by Ambache (1951), Ambache &

Edwards (1951) and Ambache & Lessin (1955) ; Burnstock *et al.* (1966) have postulated that these neurones may not be adrenergic.

Contractions of the taenia produced by nicotine and DMPP were due to an action of these drugs on intramural cholinergic ganglia for they were antagonized by ganglion-blocking drugs, tetrodotoxin, local anaesthetics and hyoscine. This conclusion agrees with that of Weis (1962) and Akubue (1966a). Neuronal nicotinic receptors were involved in the relaxation produced by nicotine or DMPP, as the response was readily antagonized by ganglion-blocking drugs, tetrodotoxin or local anaesthetics. These findings are in agreement with the observations of Weis (1962), Burnstock *et al.* (1966) and Akubue (1966b).

McN-A-343 and AHR-602 caused contraction of the taenia by acting on muscarinic receptors on the smooth muscle as the contractions were antagonized by hyoscine but not by ganglion-blocking drugs, tetrodotoxin, cinchocaine or cocaine. Previously it has been shown that McN-A-343 and AHR-602 stimulate atropine-sensitive receptors on sympathetic ganglia and thus cause pressor responses in the cat, dog and rat, which are abolished by atropine and adrenergic neurone blocking drugs but not by hexamethonium (Roszkowski, 1961 ; Levy & Ahlquist, 1962 ; Franko, Ward & Alphin, 1963 ; Smith, 1966). However, McN-A-343 does not cause pressor responses in the guinea-pig or rabbit (Smith, 1966). Our results show that both McN-A-343 and AHR-602 were, relative to acetylcholine, more effective on the taenia than has been reported with other intestinal preparations (Roszkowski, 1961 ; Franko *et al.*, 1963 ; Smith, 1966). As the concentration: response curves for these two agonists were not parallel to that for acetylcholine, exact comparisons of potency were not possible. Under optimal conditions McN-A-343 was about 20 times and AHR-602 60 times less potent than acetylcholine on a molar basis. Roszkowski (1961) had found that McN-A-343 was 200 times less active than acetylcholine on the rabbit jejunum and Franko *et al.* (1963) had reported that AHR-602 had only one-thousandth of the activity of acetylcholine on the guinea-pig ileum. In the presence of hyoscine, AHR-602 did not produce a relaxation of the taenia and McN-A-343 only occasionally caused a slow relaxation which was distinct from the rapid relaxation produced by the other agonists. The relaxation produced by McN-A-343 was not an atropine-sensitive action on intramural sympathetic ganglia as it was only observed in the presence of hyoscine. It may be similar to the sympathomimetic effect McN-A-343 produces on atropinized atria (West, Bhagat & Robinson, 1961).

Tetramethylammonium caused a contraction of the taenia by acting on muscarinic receptors on the smooth muscle as the response was antagonized by hyoscine but not by ganglion-blocking drugs, tetrodotoxin, cinchocaine or cocaine. The reduction by procaine of contractions produced by TMA (as well as by McN-A-343, AHR-602 and choline phenyl ether) might be attributed to an atropine-like activity of procaine, although it must be noted that acetylcholine was affected to a lesser extent. The response produced by TMA also sometimes contained a relaxation phase which was antagonized by ganglion-blocking drugs, tetrodotoxin or local anaesthetics and therefore represents an action on nicotinic receptors of inhibitory neurones. TMA was considered by Burn & Dale (1914) to have a nicotinic action on ganglion cells and a muscarinic action on smooth muscle ; it also has some muscarinic action at sympathetic ganglia, which is usually masked by its pronounced nicotine-like effect (Trendelenburg, 1966). On isolated tissues such as the guinea-pig tracheal chain

(Hawkins & Paton, 1958) and the guinea-pig ileum (Schild, 1960; Trendelenburg, 1961) TMA produces contractions which are unaffected by hexamethonium but are abolished by atropine. These workers concluded that the action of TMA was solely a direct muscarinic effect on the smooth muscle. In contrast, Stephenson (1956) reported that hexamethonium reduced the effect of TMA on the guinea-pig ileum.

Choline phenyl ether produced a contraction of the taenia largely by a muscarinic action on smooth muscle. In preparations with a low tone, however, the contraction was partly due to stimulation of cholinergic ganglion cells since it was reduced by ganglion-blocking drugs. In preparations with a high tone, choline phenyl ether produced only relaxation, which was changed into a contraction by ganglion-blocking drugs or tetrodotoxin. The relaxation was therefore due to an action on nicotinic receptors of inhibitory neurones. Choline phenyl ether has marked nicotine-like properties *in vivo* (Hunt & Renshaw, 1929; Hey, 1952; Edge, Mason & Wyllie, 1957) and is a potent nicotine-like agonist on isolated tissue (Edge *et al.*, 1957; Brownlee & Johnson, 1963). On the taenia it had both a muscarinic action on muscle and a nicotinic activity, whereas no such muscarine-like activity was observed with nicotine or DMPP.

TMA, choline phenyl ether, nicotine and DMPP sometimes caused a biphasic response, the contraction phase of which was complex. The contraction phase of the response to nicotine and to DMPP was sometimes not reduced by hyoscine. One explanation for this is that nicotine and DMPP were also stimulating the non-cholinergic excitatory neurones studied by Ambache & Freeman (1968). The observation that hyoscine occasionally converted contractions produced by nicotine or DMPP into biphasic responses in which the contraction phase was greater than the original contraction argues against this. Another explanation arises from the work of Day & Warren (1968) who noted that transmural electrical stimulation of kitten or rabbit ileum produced a contraction, which was converted by hyoscine into a relaxation followed by contraction. These authors suggested that hyoscine caused a temporal separation of the relaxation and contraction phases, whereas both occurred simultaneously in the absence of hyoscine. This explanation implies that an increase in the concentration of hyoscine should cause an increasing dissociation of relaxation and contraction phases. With the agonists which produced a biphasic response in experiments on the taenia, this did not occur.

The most likely explanation for the biphasic response (relaxation followed by contraction) obtained with ganglion stimulants in the presence of hyoscine is that they produce first a relaxation which in turn induces a contraction: a "rebound contraction". Transmural electrical stimulation of the taenia (Burnstock *et al.*, 1966; Campbell, 1966) and other intestinal preparations (Holman & Hughes, 1965) often results in a relaxation followed by a contraction and Bennett (1966) has suggested that this contraction may be a "rebound excitation". He observed an increase in the rate of firing of action potentials in spontaneously active cells following hyperpolarization of the taenia by transmural stimulation. The increase in the rate of firing was proportional to the degree of hyperpolarization. Burnstock & Holman (1964) have suggested that drugs also may cause hyperpolarization leading to "rebound excitation".

Biphasic responses to agonists may therefore be complex because the contraction phase could arise in three ways: first, by a direct effect on smooth muscle; second, by an action on cholinergic or other excitatory ganglia; third, by a

" rebound excitation " following relaxation. Hence, any drug which affects the relaxation phase of a response to an agonist would affect a contraction phase which involves a rebound effect. This makes it difficult to analyse at this stage in detail some of the results obtained.

Hexamethonium had no effect on the contraction phase of biphasic responses produced by TMA or choline phenyl ether in the absence of hyoscine, although it abolished the relaxation phase. In the presence of hyoscine, however, hexamethonium abolished both phases of the response to these two agonists. Therefore, contractions caused by TMA and choline phenyl ether in the presence of hyoscine are also probably " rebound contractions ".

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