Brit. J. Pharmacol. (1965), **24**, 437–448.

THE CHANGES IN RESPONSE AND IN IONIC CONTENT OF SMOOTH MUSCLE PRODUCED BY ACETYLCHOLINE ACTION AND BY CALCIUM DEFICIENCY

BY

W. D. M. PATON AND A. M. ROTHSCHILD

From the Department of Pharmacology, University of Oxford

(*Received July* 28, 1964)

The initial purpose of this paper is to describe experiments on the responses to acetylcholine of smooth muscle exposed to varying calcium concentrations, in order to throw light on the factors controlling the action of a stimulant drug. The known interaction between calcium ion concentration and the permeability of excitable tissues to sodium and potassium makes it desirable, in addition, to know the changes in ionic content of the smooth muscle when exposed to varying calcium concentration. Further, although it is well known that the action of acetylcholine causes an efflux of potassium from smooth muscle, there is little quantitative evidence as to the net change in ionic content which results. This is of importance in view of the close resemblance between the insensitivity of smooth muscle produced on the one hand by previous exposure to high doses of stimulant drugs and on the other by exposure to potassium-deficient solutions (Paton, 1961). Accordingly, determinations have also been made of the normal sodium, potassium and calcium content of the longitudinal muscle of guinea-pig ileum, and of the changes produced by acetylcholine and by calcium deprivation.

METHODS

Guinea-pigs of either sex, weighing between 450 and 600 g, were used. Ten to twenty centimetres of the terminal portion of the ileum were removed, immersed in Krebs-Ringer bicarbonate buffer and, when necessary, intraluminally flushed with several portions of fresh buffer. The contact of the external part of the gut with its discharged contents was reduced to the minimum. A strip of longitudinal muscle was prepared as described by Rang (1964). A piece of the ileum 4 to 5 cm long was cut, freed from its mesenteric attachments, and slipped, oral end leading, over a pipette having an outside diameter of 0.5 cm. A flap of the longitudinal muscle coat was freed by gently rubbing the upper few millimetres of the gut with a wad of moist cotton wool, starting at the mesenteric border and going around the upper edge to both sides. A loop of thread was firmly tied around as large a portion of the detached flap as possible and, by slightly pulling the muscle strip downwards and the remaining gut upwards, the longitudinal muscle coat could be entirely freed without being torn except at its mesenteric attachments. These operations were carried out at room temperature, care being taken to avoid the drying of the tissue. The muscle was mounted in a 4-ml. organ-bath containing Krebs-Ringer bicarbonate buffer of the following composition (mequiv/l. of glass-distilled water): Na 137; K 6.0; Mg 2.4; Ca 5.0; Cl 123; H_2PO_4 1.0; HCO_3 25; SO_4 2.0; and glucose 11.5. The bath and its fluid reservoir were bubbled with 5% carbon dioxide and 95% oxygen; the temperature was 37° C. The experiments were started 40 to 60 min after the muscle had been mounted in the bath. During this time it usually displayed an increasing amount of spontaneous activity;

this interfering effect was dependent to a large extent on stretch and it could be decreased, although usually not entirely overcome, by suitable slight reductions of its resting length. In all experiments a 1-min cycle, with exposure to drug for 10 sec and a wash-out for 3 sec, was employed. The contractions of the muscle were recorded on a smoked drum with a light auxotonic lever, magnification $\times 18$, load adjusted to either 0.30 or 0.18 g/cm deflexion on the drum according to the sensitivity of the preparation. The doses of acetylcholine quoted are in terms of the bromide salt.

Estimation of sodium, potassium and calcium in the longitudinal muscle of the guinea-pig ileum

Animals of either sex weighing 550 to 700 g were used; it was found that better results were obtained when the animals had been fasted for 24 hr so that their small intestine was nearly empty at the time of experiment, and contact of the longitudinal muscle coat with discharged faecal matter was rendered less likely. In addition, an empty intestine decreased the time required for dissection. This was performed in the manner already described. It was found that speed was an important feature in this operation: highest control values of potassium/sodium ratio, and greatest responsiveness to acetylcholine, were obtained in preparations which had only remained in the cold Krebs-Ringer medium for a maximum of 15 min. In general, six pieces of muscle were dissected from each animal; for this, 8 to 10 min elapsed between the death of the animal and the excision of the sixth piece. After dissection, each piece was mounted on suitably bent glass rods under moderate tension, and then incubated in 200 ml. of buffer at 37° C for 1 hr (" preincubation " period). When incubations with acetylcholine were to be performed in media containing low calcium, samples as well as controls were transferred to this medium for the last third of the pre-incubation period. To avoid bias caused by the position of a given piece along the gut's length or in the dissection sequence, each experimental condition was randomized by distributing it according to a Latin-square type of design. After the addition of acetylcholine, incubations were continued for 5 min. Each muscle was then removed from the bath, rapidly cut from its mountings, blotted between two layers of Whatman No. 1 filter paper, and its wet weight was measured on a torsion balance. It ranged between 15 and 6 mg. To remove organic matter, the muscles were placed in 4-ml. beakers, treated with 2 ml. of 30% hydrogen peroxide, and kept at 96° C overnight (MacIntyre, 1961). The residues were taken up in 2 to 3 ml. of 1 N-nitric acid (containing 0.1% phosphoric acid), stirred, and assayed for sodium, potassium and calcium by flame photometry using a Zeiss model PMQ II instrument. Calibration curves for each cation were made for each run of samples.

RESULTS

Dose/response relationships of acetylcholine action on the isolated longitudinal muscle of the guinea-pig ileum

Previous results (Paton, 1961) had shown that when an isometric or auxotonic lever was employed the responses obtained when increasing doses of histamine or acetylcholine were applied to the guinea-pig ileum could be represented by an equation of the type $y=x/x+k$, where y=response, x=dose of agonist and k=a constant. Similar results were obtained in the present experiments in which the response of the isolated longitudinal muscle of the ileum to acetylcholine were examined. The above expression relating dose and response can also be written as

$$1/y=1/x \,.\, k/A_m+1/A_m$$

where A_m represents maximal response. The plot of reciprocal of dose against reciprocal of response should thus result in a straight line of slope$=k/A_m$, intercept$=1/A_m$, and k_e=dose at half-maximal response. Fig. 1 represents the results of four out of a series of eight experiments in which the validity of this relationship was tested. It can be seen that a straight-line relationship between reciprocal of dose and reciprocal of response held over a ten- to twentyfold dose range. The detailed results of eight such experiments done with muscle from different animals are shown in Table 1. An average value of $k=7.7\times 10^{-10}$ g/l.

of acetylcholine bromide was obtained. The constant k has the dimensions of an equilibrium constant; its meaning will be discussed later.

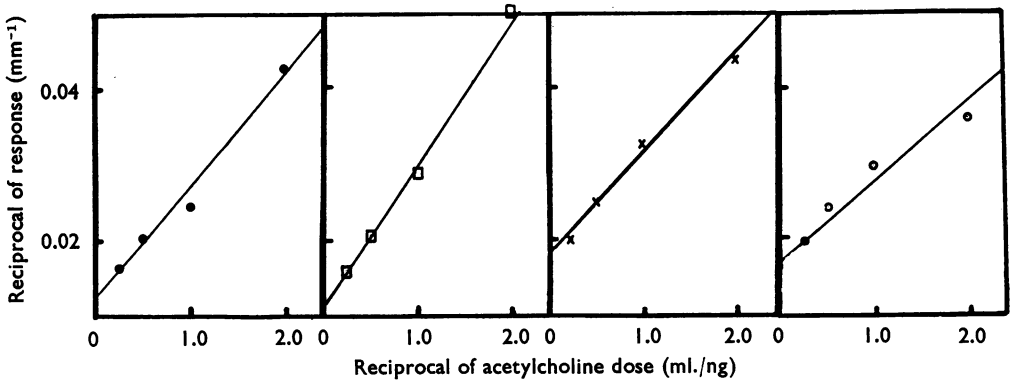

Fig. 1. Reciprocal of dose/reciprocal of response relation for acetylcholine on four different preparations of the isolated longitudinal muscle of the guinea-pig ileum. Ordinate: reciprocal of response, mm^{-1}; abscissa: reciprocal of dose of acetylcholine, ml./ng.

TABLE 1

"EQUILIBRIUM CONSTANTS" AND MAXIMAL RESPONSE FOR ACETYLCHOLINE IN NORMAL KREBS-RINGER SOLUTION

Each result was obtained with a muscle from a different animal

Experiment no.	Slope (g/ml./mm × 10^{11})	Maximal response $1/A_m$ ($mm^{-1} \times 10^2$)	Maximal response A_m (mm)	"Equilibrium constant" k (g/ml. × 10^9)
39	1·15	1·90	52·6	0·61
57	1·45	1·33	75·2	1·09
58	0·85	1·83	54·6	0·46
62	1·55	1·32	75·7	1·17
65	0·57	1·85	54·1	0·31
68	1·30	1·45	69·0	0·90
70	1·05	1·42	70·4	0·74
72	1·45	1·70	58·8	0·85
Mean ± s.e.	1·17 ± 0·12	1·60 ± 0·15	63·7 ± 6·1	0·77 ± 0·11

Effect of partial calcium deficiency on the values of k *and* A_m

Calcium clearly plays an important role in controlling the response of smooth muscle to stimulants such as acetylcholine (Durbin & Jenkinson, 1961; Edman & Schild, 1962; Bülbring & Kuriyama, 1963). But a quantitative investigation as to how a limited calcium deficiency affects the dose/response curve of a stimulant acting on smooth muscle appears to be lacking. The calcium concentrations employed in our experiments, 1 mM and 0.5 mM, allowed reproducible and stable responses to acetylcholine which, save for having sometimes a slower time course, could not be distinguished qualitatively from responses obtained with the normal 2.5 mM concentration. Fig. 2 shows, for muscle preparations exposed to 0.5 mM-calcium, that the fall in sensitivity to acetylcholine is fully developed in 10 min, and is maintained for 2 hr at least. The experiments to be described all began after a 20-min equilibration period in the low calcium medium, and were mostly completed within 2 hr.

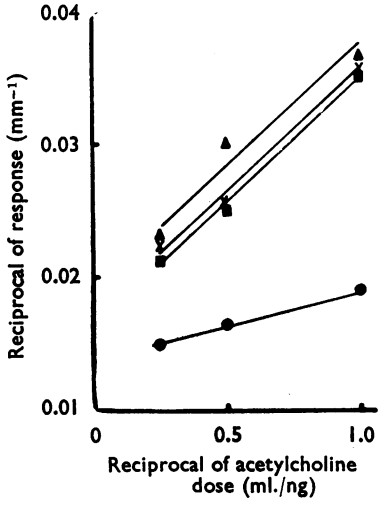

Fig. 2. Effect of time in low-calcium (20%) Krebs-Ringer solution on the responsiveness to acetylcholine. ●——●, Control responses (normal Krebs-Ringer); ■——■, ×——×, and ▲——▲, responses after 10, 40 and 120 min respectively in low calcium. Ordinate: reciprocal of response, mm^{-1}; abscissa: reciprocal of dose of acetylcholine, ml./ng.

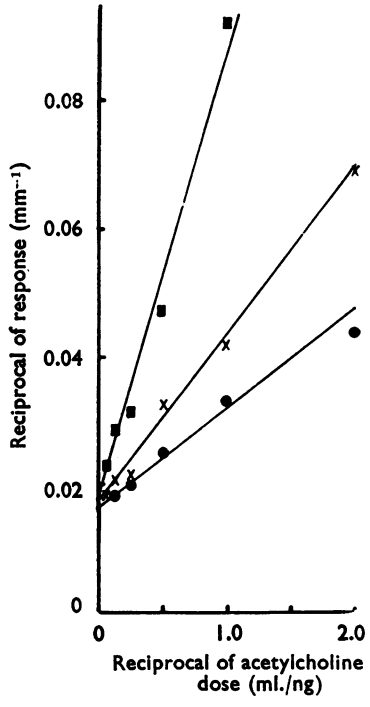

Fig. 3. Dose/response relation for acetylcholine in normal (●——●), 60% calcium-deficient (×——×) and 80% calcium-deficient (■——■) Krebs-Ringer solution. Ordinate: reciprocal of response, mm^{-1}; abscissa: reciprocal of dose of acetylcholine, ml./ng.

TABLE 2

"EQUILIBRIUM CONSTANTS" AND MAXIMAL RESPONSE FOR ACETYLCHOLINE IN CALCIUM-DEFICIENT KREBS-RINGER MEDIUM

*As percentage of that present in normal Krebs-Ringer bicarbonate buffer. Each result was obtained with a muscle from a different animal

Experiment no.	Calcium content (%)*	Slope (g/ml./mm × 10^{11})	Maximal response $1/A_m$ ($mm^{-1} \times 10^5$)	Maximal response A_m (mm)	"Equilibrium constant" k (g/ml. × 10^9)
39	40	5·30	1·80	55·6	2·94
62	40	2·15	1·32	75·7	1·63
65	40	1·90	1·95	51·4	0·98
68	40	2·80	1·45	69·0	1·93
	Mean ± s.e.	3·04 ± 0·78	1·63 ± 0·15	62·9 ± 5·6	1·87 ± 0·41
57	20	4·50	1·55	64·5	2·90
70	20	5·80	1·65	60·6	3·50
72	20	7·23	1·70	58·8	4·25
	Mean ± s.e.	5·84 ± 0·80	1·63 ± 0·02	61·3 ± 3·2	3·55 ± 0·40

Fig. 3 illustrates the changes in the reciprocal dose/response relationship produced by changes in the calcium concentration of the medium. The relationship is still linear; the lowering of the calcium concentration caused a pronounced increase of the slope of the lines but only in a small increase of the $1/A_m$ values. Table 2 summarizes the results of seven such experiments. The differences from the normal value of $1/A_m$ at both 1.0 mM and 0.5 mM calcium levels were too small to be statistically significant. In contrast, lowering the concentration of calcium significantly affected the values for k. Fig. 4 shows the relation between $1/k$ and calcium concentration obtained from the experiments in Tables 1 and 2.

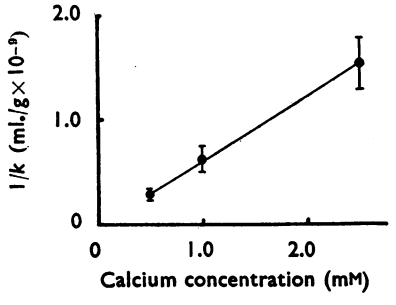

Fig. 4. Effect of calcium concentration on the reciprocal of the equilibrium constant for the action of acetylcholine on the longitudinal muscle of the guinea-pig ileum. Values of $1/k$ are derived from the experimental data shown in Tables 1 and 2 and are presented with the standard errors of their means. Ordinate: $1/k$ (ml./g×10^{-9}); abscissa: calcium concentration (mM).

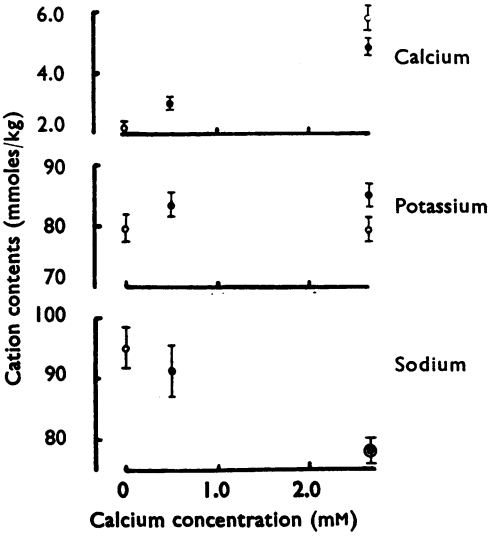

Fig. 5. Effect of a 20-min incubation period in calcium-deficient Krebs-Ringer medium on the sodium, potassium and calcium contents of the longitudinal muscle of the guinea-pig ileum. Results obtained from data in Table 3. ○ and ● denote averages obtained from two groups of eight and twelve samples of muscle respectively. Ordinate: mmoles of respective cation per kg wet weight of tissue; abscissa: calcium concentration (mM).

The effect of acetylcholine and of varying calcium concentration on sodium, potassium and calcium content of the longitudinal muscle

Although several reports on the effect of acetylcholine or its analogues on ^{42}K efflux have been published (Born & Bülbring, 1956; Durbin & Jenkinson, 1961; Weiss, Coulson & Hurwitz, 1961; Bannerjee & Lewis, 1964); no measurements of the net change of sodium or potassium content due to acetylcholine have yet been described for smooth muscle of mammalian origin. Such changes could be neatly demonstrated in the isolated longitudinal muscle strip from the guinea-pig ileum. The first part of Table 3 shows the results of incubating control samples and samples in the presence of 2 μg/ml. of acetylcholine in normal buffer for 5 min at 37° C. The treatment with acetylcholine resulted in a

TABLE 3

EFFECT OF ACETYLCHOLINE ON POTASSIUM, SODIUM AND CALCIUM CONTENTS OF THE ISOLATED LONGITUDINAL MUSCLE OF THE GUINEA-PIG ILEUM IN NORMAL AND CALCIUM-DEFICIENT MEDIA

Unless indicated, comparisons were found to be insignificant ($P>0.05$). Acetylcholine, 2 μg/ml. for 5 min at 37° C. Numbers in parentheses denote the numbers of samples of muscle analysed. The figures are mean values, with standard errors

	Cation contents (mmoles/kg) of					
	Potassium		Sodium		Calcium	
Medium	Controls	Acetylcholine	Controls	Acetylcholine	Controls	Acetylcholine
Normal	85·0±2·1 (15)	74·9±1·9 (15)	78·1±1·7 (15)	85·3±2·1 (15)	4·84±0·13 (15)	4·64±0·14 (15)
	$P=0.01$		$0.05>P>0.02$		$P<0.001$	$P<0.001$
20% calcium	83·4±2·1 (12)	73·5±2·8 (12)	91·3±4·0 (12)	96·4±4·9 (12)	3·01±0·18 (12)	3·32±0·32 (12)
	$P=0.01$					
Normal	79·3±1·6 (8)	—	77·7±3·2 (8)	—	5·79±0·35 (8)	—
			$P<0.005$		$P<0.001$	
No calcium	79·5±1·6 (8)	65·8±1·2 (8)	94·8±3·5 (8)	98·7±2·9 (8)	2·22±0·15 (8)	1·92±0·15 (8)
	$P<0.001$					

significant loss of 10.1 mmoles (11.9%) of potassium, and gain of 7.2 μmoles (9.2%) of sodium, per kg wet weight of tissue. In contrast to these changes, no significant effect on the calcium content of the muscle was detected.

The effects of an 80% decrease of the calcium content of the incubation medium as well as of the complete absence of this cation on acetylcholine action and ionic contents were next investigated (Fig. 5 and Table 3). The mere placing of the muscle in a low calcium medium had a profound effect not only on tissue calcium, which was decreased by 1.83 mmoles/kg (38%), but also on sodium content, which was increased by 13.2 mmoles/kg (16.9%). Potassium levels were unaffected, remaining the same as those found in the controls. In the calcium-free medium, calcium content was further reduced and sodium content further increased, but again potassium levels were unchanged. It may be noted that the control values for potassium in the two groups of experiments were different; in one, the mean of fifteen determinations was 85.0±2.1 mmoles/kg; in the other, the mean of eight determinations was 79.3±1.6 mmoles/kg. The difference was not statistically significant. The relation between calcium and sodium levels is brought out in Fig. 5; they vary inversely as the calcium concentration of the bathing medium changes.

Acetylcholine in a medium containing 0.5 mM-calcium released the same amounts of potassium as in the normal medium, namely 9.9 mmoles/kg (11.8%). In the absence of calcium the drug's effect was apparently increased, a loss of 13.7 mmoles/kg (17.3%) being found. The increase in sodium content due to acetylcholine was, however, much less pronounced with calcium deficiency, averaging 5.1 mmoles/kg (5.6%) in 0.5 mM-calcium and 3.9 mmoles/kg (4.1%) in the calcium-free medium; in neither case was this effect statistically significant. It appears from the results of Table 3, therefore, that acetylcholine reduced muscle potassium to about the same extent regardless of the tissue content of calcium or sodium, and regardless of the muscle's mechanical response to stimulation which must have been much reduced in the calcium-deficient media.

TABLE 4

EFFECT OF DOSE OF ACETYLCHOLINE ON THE CHANGES OF POTASSIUM AND SODIUM CONTENT OF THE ISOLATED LONGITUDINAL MUSCLE OF THE GUINEA-PIG ILEUM

Exposure to acetylcholine was for 5 min at 37° C. The first two rows give control values without acetylcholine. Figures in brackets indicate numbers of experiments performed

Dose of acetylcholine (μg/ml.)	Sodium (mmoles/kg)	Potassium (mmoles/kg)
0	78·1±1·7 (15)	85·0±2·1 (15)
0	77·7±3·2 (8)	79·3±1·6 (8)
0·04	82·9±4·6 (4)	82·1±4·1 (4)
0·20	84·7±2·8 (4)	81·9±4·6 (4)
2·00	85·3±2·1 (15)	74·9±1·9 (15)
100·00	95·2±4·3 (4)	66·6±2·3 (4)

A further experiment was done to test whether the 2 μg/ml. concentration of acetylcholine used represented a maximal stimulus for potassium loss. Table 4 shows that this is not so. A fiftyfold increase of the dose of acetylcholine was able to raise potassium loss to about 20% and sodium gain to 22%. Lower doses of acetylcholine (0.2 and 0.04 μg/ml.) were also tested but the changes in ionic content were now too small to be significant.

Calcium deficiency and desensitization

Evidence indicating the altered state of responsiveness of smooth muscle to acetylcholine could also be obtained in another way. This was done by estimating the susceptibility of the muscle to nonspecific desensitization by acetylcholine (Cantoni & Eastman, 1946; Paton, 1961). For the purpose of this study the extent of desensitization evoked by a relatively high conditioning dose of acetylcholine was assessed by measuring the extent to which the response to a smaller, test dose of the drug was depressed shortly after wash-out of the desensitizing dose. The results of such experiments are illustrated in Table 5. The

TABLE 5

EFFECT OF CALCIUM DEFICIENCY ON THE DESENSITIZING EFFECT OF ACETYLCHOLINE

Desensitizing doses were given for 5 min. Test doses were applied 1 min after wash-out of desensitizing dose. The dose ratio was obtained by dividing the test dose by the dose which gave equal contraction of the muscle before desensitization. The calcium-deficient buffer solution contained 20% of its normal content of calcium

Experiment no.	Desensitizing dose (ng/ml.)	Test dose (ng/ml.)	Dose ratio	
			Normal	Ca-deficient
112	2,000	50	27	9
116	2,000	50	32	14
98	40	5	18	3
100	40	5	3	2
98	20	5	8	3
100	20	5	2	1

intensity of desensitization is expressed as a dose-ratio, that is the ratio by which a test dose of the stimulant had to be increased in order to match a control response obtained before desensitization.

Desensitization by acetylcholine still occurred under conditions of calcium deficiency, but it was less intense, and correspondingly the tissue returned to its initial state sooner. Comparing this result with the ionic changes produced by acetylcholine normally and in a

low calcium medium, the desensitization correlates more closely with the gain in sodium (which was reduced in calcium deficiency) than with the loss in potassium. This suggests that the reduced desensitization in calcium deficiency may be associated with a lesser amount of sodium to be extruded by the tissue in order to return to its initial state before treatment with acetylcholine.

DISCUSSION

The dose/response curve for acetylcholine

At first sight the result of these experiments, that calcium deficiency increases the apparent equilibrium constant for the response to acetylcholine, may seem to conflict with that of the preceding paper (Paton & Rothschild, 1965), where it was found that similar conditions of calcium deficiency did not alter the equilibrium constant of hyoscine, but only the rate constants. Such a view disregards the radical difference between the conditions in the two sets of experiments. In the latter, using the method of the " dose-ratio," the response of the muscle strip is kept constant, so that the changes in sensitivity that take place are all attributable to reaction with the antagonist drug and not to nonspecific desensitization. But in the former case, a dose/response curve for a stimulant can only be obtained by evoking varying responses from the tissue, each associated with varying degrees of desensitization. It has been argued elsewhere that, since this desensitization is greater the sooner the test for it is made after the exposure to the desensitizing dose, it must be supposed that *during* the action of a stimulant, desensitization is present. The magnitude of the desensitization can be so great (100-fold or more is readily obtained), that it must dominate the shape of the dose/response curve as soon as doses are used substantially above threshold. The " equilibrium constant " cannot, therefore, simply measure the acetylcholine-receptor interaction but must (for other than near-threshold doses) be a measure of the responsive capacity of the tissue to drug-receptor interaction.

At the same time, it was found previously, and confirmed again in this study, that, if a method of recording is used which responds to tension developed (auxotonic or isometric recording) rather than measuring shortening at constant load (isotonic recording), the dose/response relation is well fitted by the Langmuir isotherm. It may be suggested that there are no grounds for preferring one method of recording to another. There are, however, two reasons for believing that auxotonic or isometric recording will reflect the development of the active state in the muscle more closely than isotonic recording. First, with the former the muscle in its resting state can be loaded as lightly as desired; but with isotonic recording a definite and quite substantial load is required if the contraction during a maximal response is to stay on the smoked drum, or is to stay within the range of linear recording by the lever. This means that under isotonic conditions the movement of the lever will not begin until the muscle generates a tension at least equal to that of the resting load. As a result a " toe " develops to the dose/response curve (see Paton, 1961, Fig. 3) which steepens the subsequent part of the curve. (It is for this reason that isotonic recording is often preferable for a discriminating biological assay.) This distortion is avoidable by methods which record tension. The second reason depends on the assumption that the best method would be that which comes closest to measuring the active state set up in the muscle. The active tension of a muscle varies with its length, being maximal at or near *in vivo* resting length. With isotonic recording, the active tension is measured at varying length, so that the response is

determined not only by the intensity of the active state but also by the relationship between length and tension. With isometric recording, the function relating tension to length is not involved, or only within the limits of the compliance of any series elastic component. Auxotonic recording allows more shortening; but with both methods the length of the preparation can be kept close to resting length.

We accept therefore, a Langmuir relationship between dose of acetylcholine and recorded response. But it was pointed out above that the existence and magnitude of desensitization implies that the limit to the response is set not by drug-receptor interaction but by capacity of the tissue to respond. In that case, even at maximal response, the receptors are far from saturated; it has been pointed out elsewhere that the existence of nonspecific densensitization entails the appearance of the phenomenon of " spare receptors." But if the receptors are far from saturation, then during the action of an agonist such as acetylcholine we shall be dealing with the initial linear phase of the drug-receptor interaction, where receptor occupation or activity is directly proportional to drug concentration. It would then follow that the nonlinear shape of the observed dose/response describes the relation between the chemoceptive stimulus and the response: that is, that the " capacity to respond " depends on a saturable reaction, approximated by a Langmuir isotherm.

This result may be stated more generally, in the hypothesis that the final response of the tissue depends on two processes in sequence, both of Langmuir type. Making the simplest assumptions, the response of the tissue would be given by

$$y = \left[A_\varrho \cdot \frac{k_2 x}{x + k_2/k_1}\right] \Big/ \left[\frac{k_2 x}{x + k_2/k_1} + k_\varrho\right]$$

where equilibrium is assumed to occur, k_2 and k_1 are rate constants for the drug-receptor reaction, k_ϱ is the equilibrium constant for the second-stage reaction, and A_ϱ is the maximum response.

Rearranging, we obtain $y = \{(A_\varrho k_2)/(k_2 + k_\varrho)\}\ [x/\{x + (k_\varrho k_2/k_1)/(k_2 + k_\varrho)\}]$.

This shows that, independently of the actual values of k_1, k_2 and k_ϱ, the function resulting from two (or more) Langmuir functions in sequence is also a Langmuir function, and that for two functions the " equilibrium constant " is $k = (k_\varrho k_2/k_1)/(k_2 + k_\varrho)$.

In the earlier investigations it was noted that the fact that a dose/response curve can be fitted by a Langmuir isotherm might be simply fortuitous. The present analysis suggests that this may be too pessimistic, and that it may be, in fact, an expression of the nature of the successive stages connecting drug-receptor reaction with final response. This approach offers a means of analysing desensitization more completely, which will be developed in another paper. For the present, two simple cases may be considered. First, if k_2 is small, that is the drug is primarily an antagonist, then $k = k_2/k_1$; thus, for a feeble stimulant, the drug-receptor reaction is dominant. But if k_2 is large, and the drug is primarily a strong stimulant, then $k = k_\varrho/k_1$. This is an interesting result, for it shows that, under the conditions where a strong stimulant is used, and if a Langmuir model for the link between chemoceptive and mechanical stages in the response is assumed, then it is the association rate constant rather than the equilibrium constant of the drug-receptor reaction that determines the final response.

We suggest, therefore, that the equilibrium constant obtainable from a dose/response curve does not represent the equilibrium constant of the drug-receptor reaction, and so does

not measure the drug's affinity, but is a function of the forward rate constant and of the response capacity of the tissue. On this interpretation the influence of calcium deficiency on the action of hyoscine is similar to that on the action of acetylcholine, namely an interference with the rate of drug-receptor association.

Change in ionic content

The ionic composition of the longitudinal strips is comparable with that described for other types of smooth muscle. The sodium content (78 mM/kg) is lower and the potassium content (83 mM/kg) higher than the original estimates for taenia coli (sodium, 103 mM/kg; potassium, 52 mM/kg), but the most recent values (sodium, 84 mM/kg; potassium, 90 mM/kg; Goodford, 1964) hardly differ significantly. Estimates of the extracellular space have not been made for the longitudinal muscle. If Goodford's (1964) estimate of the extracellular fraction of taenia as 0.45 applies, the intracellular ionic concentrations are sodium, 31 mM/kg and potassium, 145 mM/kg. But, in the light of the pattern of isotopic exchange for taenia, and of the electronmicroscopic appearance of the longitudinal muscle, it seems unlikely that ionic movements and concentrations can be discussed in terms only of two phases (intracellular and extracellular). The true intracellular concentrations, and hence the concentration gradients, must therefore be regarded as doubtful, so that changes in ionic content can at present only be discussed qualitatively. No measurements of ion flux were made, so that the possibility exists that balanced changes of influx and efflux occurred, which left the ionic contents unaltered.

The effect of calcium deficiency on ionic content was not altogether as expected. Frankenhauser & Hodgkin (1957) showed that a fivefold reduction of the calcium content of sea water bathing a squid axon caused an increased permeability to sodium and potassium corresponding to that produced by a membrane depolarization of 15 mV. In our experiments on the longitudinal muscle, however, even total calcium deprivation (which produced a 50% fall in calcium content) did not change the potassium content significantly, although sodium content rose substantially, by about 20%. These results suggest that calcium may be important in controlling the sodium extrusion mechanism in smooth muscle. It must be noted, however, that in a number of tissues (see Hodgkin, 1958) there is evidence that the sodium extrusion mechanism was coupled to an inward potassium movement; possibly calcium is necessary for the normal co-ordination of movement of these two ions. The fall in calcium content is similar to that reported by Schatzmann (1961) who found that about 65% of the calcium in guinea-pig taenia coli consisted of a non-exchangeable fraction, not to be removed even by prolonged exposure to a calcium-free medium. It is probable, therefore, that it is a fall in the freely exchangeable portion of the calcium which is responsible for the marked effect on sodium content.

Acetylcholine, under normal conditions, lowered potassium content and raised the sodium content by approximately equal amounts, and the magnitude of the change was graded with dose. Calcium content was not appreciably affected. With low calcium, however, a dissociation between changes in potassium and sodium content again appeared; acetylcholine produced about the same fall in potassium content as before, but the rise in sodium was so far reduced as no longer to be significant. Under no conditions did acetylcholine alter calcium content. Of the changes in ionic composition, then, it is a loss in potassium that is most consistently produced by acetylcholine, and this loss is a substantial one,

approaching 30 mM/kg with a high dose of acetylcholine, if an extracellular fraction of 0.45 is assumed.

The intensity of desensitization, after a conditioning dose of acetylcholine, did not correlate with changes in potassium or calcium content; but it corresponded with the change in sodium content, again a substantial one, produced by the acetylcholine. This may mean simply that the sodium extrusion mechanism is electrogenic in smooth muscle, and its increased activation leads to outward current which reduces the depolarization produced by acetylcholine; or it may reflect a more radical interference by intracellular sodium with the chemoceptive action of acetylcholine.

SUMMARY

1. In the presence of reduced calcium concentrations, the maximal response of guinea-pig ileum longitudinal muscle is little changed, but the apparent equilibrium constant (k) of the dose/response curve is increased. Theoretical reasons are given for the view that k reflects the association rate constant of the drug-receptor reaction rather than a true equilibrium constant.

2. Under the action of acetylcholine in normal media, the potassium content of the tissue may fall by up to 16 mM/kg, with a quantitatively similar rise in sodium content. No change in calcium content was detected.

3. With calcium deficiency or deprivation, the sodium content rises, the calcium content falls, and the potassium content is unchanged. Treatment with acetylcholine now causes approximately the same potassium loss as normally, but sodium gain is reduced.

4. The desensitization produced by previous exposure to a high dose of acetylcholine is reduced by calcium deficiency. It appears to be related not to changes in calcium or potassium content, but to the gain in sodium.

Financial assistance to A. M. R. by C.A.P.E.S. (Brazil) and the Brazilian Research Council, Rio de Janeiro, is gratefully acknowledged. A. M. R. was British Council Bursar. We are indebted to Dr R. Casteels for performing the flame photometry measurements for us.

REFERENCES

BANERJEE, A. K. & LEWIS, J. J. (1964). Effects of smooth muscle stimulants and their antagonists upon potassium ion uptake and release in strips of guinea-pig ileum. *J. Pharm. Pharmacol.*, **16**, 134–136.

BORN, G. V. R. & BÜLBRING, E. (1956). The movement of potassium between smooth muscle and the surrounding fluid. *J. Physiol. (Lond.)*, **131**, 690–703.

BÜLBRING, E. & KURIYAMA, H. (1963). Effects of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea-pig taenia coli. *J. Physiol. (Lond.)*, **166**, 59–74.

CANTONI, G. L. & EASTMAN, G. (1946). On the response of the intestine to smooth muscle stimulants. *J. Pharmacol. exp. Ther.*, **87**, 392–399.

DURBIN, R. P. & JENKINSON, D. H. (1961). The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions. *J. Physiol. (Lond.)*, **157**, 74–89.

EDMAN, K. A. P. & SCHILD, H. O. (1962). The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. *J. Physiol. (Lond.)*, **161**, 424–441.

FRANKENHAUSER, B. & HODGKIN, A. L. (1957). The action of calcium on the electrical properties of squid axones. *J. Physiol. (Lond.)*, **137**, 218–244.

GOODFORD, P. J. (1964). Chloride content and ^{36}Cl uptake in the smooth muscle of the guinea-pig taenia coli. *J. Physiol. (Lond.)*, **170**, 227–237.

HODGKIN, A. L. (1958). Ionic movements and electrical activity in giant nerve fibres. *Proc. roy. Soc. B*, **148**, 1–37.

MACINTYRE, I. (1961). Flame photometry. *Advances in Clinical Chemistry*, **4**, 1–28.

PATON, W. D. M. (1961). A theory of drug action based on the rate of drug-receptor combination. *Proc. roy. Soc. B*, **154**, 21–69.

PATON, W. D. M. & ROTHSCHILD, A. M. (1965). The effect of varying calcium concentration on the kinetic constants of hyoscine and mepyramine. *Brit. J. Pharmacol.*, **24**, 432–436.

RANG, H. P. (1964). Stimulant actions of volatile anaesthetics on smooth muscle. *Brit. J. Pharmacol.*, **22**, 356–365.

SCHATZMANN, H. J. (1961). Calciumaufnahme und Abgabe am Darmmuskel des Meerschweinchens. *Pflugers Arch. ges. Physiol.*, **274**, 295–310.

WEISS, G. B., COULSON, R. E. & HURWITZ, L. (1961). K transport and mechanical responses of isolated longitudinal smooth muscle from guinea-pig ileum. *Amer. J. Physiol.*, **200**, 789–793.