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EVIDENCE THAT HISTAMINE AND CARBACHOL MAY OPEN THE SAME ION CHANNELS IN LONGITUDINAL SMOOTH MUSCLE OF GUINEA-PIG ILEUM

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SUMMARY

1. Membrane potential was recorded intracellularly by micro-electrode in separated longitudinal muscle of guinea-pig ileum. Electrotonic potentials were evoked in longitudinal strips by passing current between large external electrodes in the partition chamber.

2. Histamine increased the frequency of action potential discharge at low concentrations and depolarized the membrane. At higher concentrations it caused substantial depolarization and action potential discharge was abolished. Carbachol had similar actions but the maximal depolarization by carbachol (using 10^{-4} M) was some 4–5 mV greater than maximal depolarization by histamine (using 10^{-4} M).

3. The change in size of evoked electrotonic potentials was used to estimate the effects of carbachol and histamine on the conductance of the smooth muscle membrane. The equilibrium potentials for histamine and carbachol depolarizations were estimated from their relative effects on potential and conductance and were found to be not significantly different; measurements of the effects on conductance showed that 10^{-4} M-histamine increased conductance about 8-fold whilst 10^{-4} M-carbachol had a much greater effect on conductance. This difference could explain the differing maximal depolarizing effects of these agents if both were assumed to open channels having the same ionic selectivity (i.e. equilibrium potential).

4. The efflux of ^{42}K was studied in separated strips of longitudinal ileal muscle from guinea-pig. In the presence of a concentration of carbachol (2×10^{-5} M or 10^{-4} M) having a maximal effect on ^{42}K efflux rate, histamine (10^{-4} M) did not increase efflux further although 120 mM-potassium did so. Experiments with the irreversible muscarinic receptor blocker, propylbenzilylcholine mustard, indicated that the number of muscarinic receptors did not limit the ^{42}K efflux response to carbachol and it was suggested that the response was limited by the availability of ion channels which could be opened by activated muscarinic receptors.

5. Contractions to histamine and carbachol in 120 mM-potassium depolarizing solution were followed upon washing by a relaxation below basal tension. Carbachol, but not histamine, showed a pronounced and long lasting secondary contraction following this relaxation.

6. These results are consistent with the idea that activated histamine and activated muscarinic receptors open the same ion channels in the smooth muscle

membrane to produce depolarization, increased action potential discharge and contraction, although muscarinic receptors can open more of these. However, there was evidence that the opening of these channels is not the only pathway between receptor activation and contraction.

INTRODUCTION

In smooth muscles which freely generate action potentials, modulation of action potential discharge is probably the most important mechanism of varying tension. In contrast to other mechanisms by which stimulant transmitters and other stimulant substances increase tension, such as increasing the calcium permeability of the cell membrane directly or releasing calcium from bound sites within the cell, the action potential can propagate and generate tension in cells remote from that in which it originated (e.g. see Bolton, 1979). It may be that stimulants such as acetylcholine can act specifically on potential-sensitive ion channels so increasing action potential discharge (Bolton, 1971, 1975) but normally a major mechanism by which action potential discharge is accelerated is by means of depolarization of the cell membrane. In the case of acetylcholine this depolarization seems to involve an increase in the permeability of the cell membrane to sodium, potassium and possibly calcium ions (Durbin & Jenkinson, 1961; Bolton, 1972, 1973, 1979).

In the preceding paper (Bolton & Clark, 1981*b*) it has been described how histamine and carbachol produce similar maximal contractions of longitudinal smooth muscle of guinea-pig ileum. Both histamine and carbachol, acting via separate receptors, increase the efflux of potassium across the smooth muscle cell membrane (Born & Bülbring, 1956; Banerjee & Lewis, 1964; Burgen & Spero, 1968; Banerjee, 1972), although the maximal effect of histamine is less than the maximal effect of carbachol (Bolton & Clark, 1981*b*). It is known that in longitudinal smooth muscle of the taenia of the guinea-pig caecum, histamine depolarizes the membrane and increases the frequency of action potential discharge (Bülbring, 1954, 1955, 1957; Bülbring & Burnstock, 1960). Thus the actions of histamine and carbachol on longitudinal intestinal smooth muscle show a number of similar features. At some point between contraction and the activation of histamine receptors by histamine, or the activation of muscarinic receptors by carbachol, the pathways by which these two types of activated receptor are linked to contraction must converge. Evidence is presented here that activated histamine receptors and activated muscarinic receptors may act to open ion channels with the same ionic selectivity and from the same population.

METHODS

Guinea-pigs (300–700 g) of either sex were stunned, bled and a portion of ileum removed. Strips of longitudinal muscle (3–5 mm wide, 1–2 cm long) were cut and separated from the underlying circular muscle (Bolton, 1972; Bolton & Clark, 1981*a*, *b*).

Electrophysiological experiments

Intracellular recordings were made at 35 °C from small (5 × 5 mm) portions of longitudinal strips using glass microelectrodes filled with 3 M-KCl and having resistances of 30–80 MΩ as described previously (Bolton, 1972). In some experiments large extracellular silver-silver chloride plate electrodes were used to elicit electrotonic potentials in a partition chamber bath similar to that

described by Abe & Tomita (1968) (Bolton, 1972). In a few experiments polarization was applied instead by means of a suction electrode (made from plastic tubing, o.d. 1 mm) applied to the surface of the strip and smooth muscle cells penetrated close to this.

Radioisotope experiments

Longitudinal ileal strips were first incubated in a physiological salt solution (at 37 °C) with radioactive ^{42}K for 3–5 hr. Following loading the strips were washed in a non-radioactive solution and then continuously perfused with warm physiological solution (2·2 ml./min) one end of the strip being attached to an isotonic transducer. Both loading and perfusing solutions contained 2×10^{-7} M-tetrodotoxin. The determination and calculation of the changes in the loss ^{42}K and of muscle shortening in response to drug application have been previously described by Bolton & Clark (1981*a*, *b*).

Solutions

A modified Krebs solution was used of the following composition (mM) Na 137$^+$; K$^+$ 5·9; Ca^{2+} 2·5, Mg^{2+} 1·2; Cl$^-$ 134, HCO^-_3 15·4, H_2PO_4 1·2, glucose 11·4. This solution was gassed with 95% O_2:5% CO_2 and had a pH of about 7·2. In the radioisotope experiments drug was applied by transferring the inflow tube to a physiological salt solution containing it. In the electrophysiological experiments rapid application of drugs could be achieved by switching the bathing solution to a similar solution containing the drug. This necessitated keeping the drug dissolved in physiological salt solution for several hours at 35 °C and pH 7·2. In some experiments where drug-induced contractions were elicited in high potassium-depolarizing solution, a solution of 120 mM-potassium and 22 mM-sodium was used, the composition being otherwise unchanged.

Drugs

Atropine sulphate (Sigma), carbachol chloride (BDH), cimetidine (a gift from SKF), histamine acid phosphate (BDH), mepyramine maleate (a gift from May and Baker Ltd.), ouabain (BDH), phentolamine methane sulphonate (CIBA) propranolol hydrochloride (ICI) propylbenzilylcholine mustard bromide (kindly given to us by Dr E. C. Hulme), prostaglandin E_2 (Upjohn), tetrodotoxin (Sigma). Propylbenzilylcholine mustard was dissolved in dehydrated absolute alcohol. A dilution to 10^{-5} M was made into 16 mM-phosphate buffer at pH 7·6 and cyclization to the ethyleniminium form allowed to occur for 30 min before further dilution and application to the muscle in physiological salt solution (cf. Gill & Rang, 1966; Burgen, Hiley & Young, 1974).

RESULTS

Depolarizing action of histamine.

Histamine increased the frequency of action potential discharge in longitudinal muscle of guinea-pig ileum (Fig. 1) as in taenia (Bülbring & Burnstock, 1960). If discharge was in bursts, each burst occurring on a slow potential, then histamine increased the frequency of the bursts. Concentrations above 10^{-7} M produced appreciable depolarization and if this was more than 30 mV then action potential discharge ceased (Figs. 1, 3). These actions of histamine are very similar to those of acetylcholine or carbachol on this muscle. A quantitative study of the electrophysiological effects of histamine was made. The relationship between depolarization and concentration of histamine is shown in Fig. 2 where it is compared with data obtained previously (Bolton, 1972, 1973) for carbachol. The depolarizing actions of histamine and carbachol below about 10^{-6} M were very similar but higher concentrations of histamine seemed slightly less effective in producing large depolarizations. This point was studied specifically in a number of further experiments in which a maximally effective depolarizing concentrations (10^{-4} M) of carbachol and histamine were compared alone or in combination on the same muscle (Fig. 3). Carbachol applied

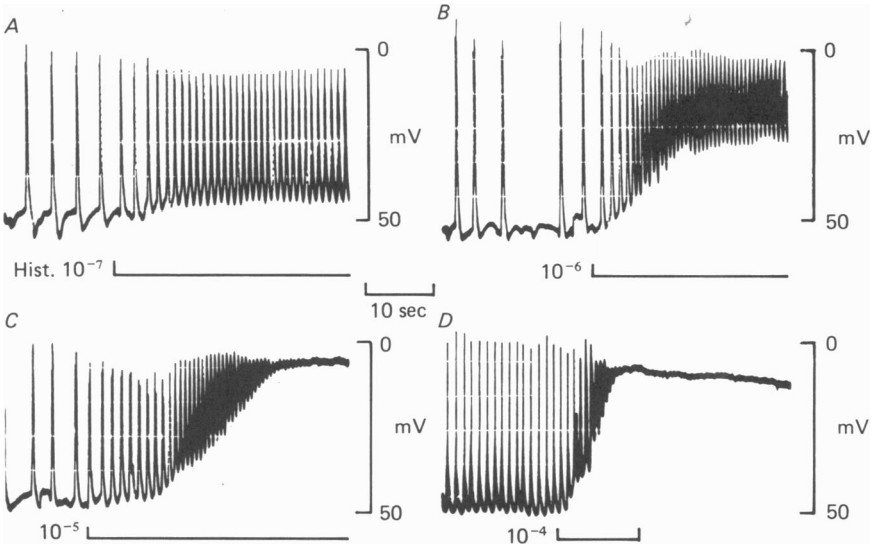

Fig. 1. Action of histamine on the electrical activity of longitudinal ileal muscle. Histamine 10^{-7} M (*A*), 10^{-6} M (*B*), 10^{-5} M (*C*) and 10^{-4} M (*D*) was present in the solution bathing the muscle for the periods indicated by the horizontal lines.

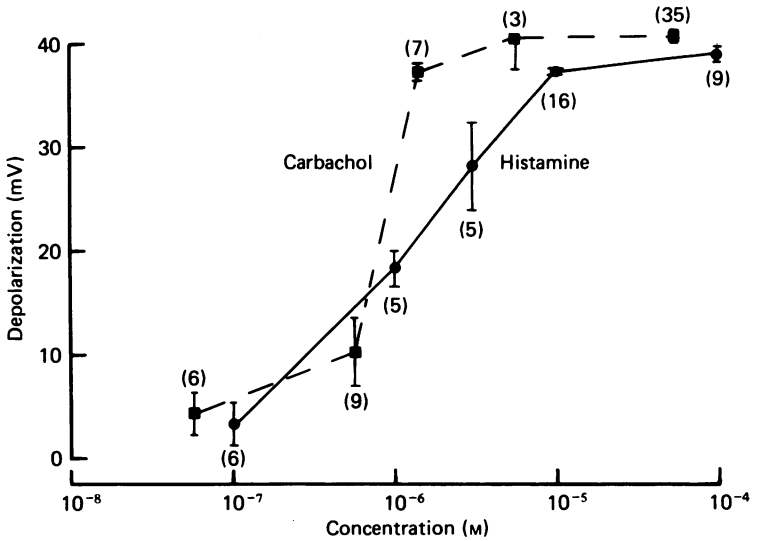

Fig. 2. Relationship between depolarization and the concentration of histamine (●). Each point is the mean ± S.E. of the mean of the indicated number of results. Before averaging, depolarizations were scaled by a factor $50/x$ where x was the membrane potential before the application of histamine. Also shown (■) is the relationship between scaled depolarization and concentration of carbachol obtained by Bolton (1972, 1973) on separated longitudinal ileal muscle under the same conditions.

in the presence of histamine produced about 5 mV more depolarization; histamine applied in the presence of carbachol was without effect on membrane potential. Also, the depolarization produced by histamine tended to decline slightly during its application, and to be less if histamine was reapplied (Fig. 3*C*).

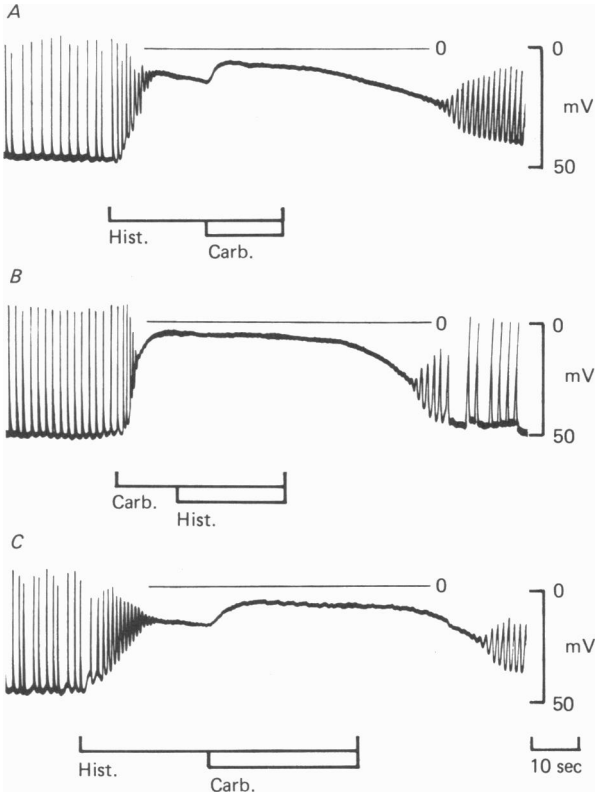

Fig. 3. *A–C*, effects of maximally effective depolarizing concentrations of histamine and carbachol alone and in combination. Histamine (10^{-4} M, Hist.) or carbachol (10^{-4} M, Carb.) were present in the bathing solution for the indicated periods. The three records were obtained from the same cell at about 10 min intervals. Depolarization by histamine is increased if carbachol is added, but depolarization by carbachol is not increased if histamine is added; the difference in their maximal depolarizing effects is about 5 mV. Notice that the response to the third application of histamine (*C*) is reduced compared to the first (*A*).

Theory

If it is supposed that activation of muscarinic receptors and of histamine receptors opens ion channels with the same ionic selectivity (both types of receptors may for example act to open ion channels from the same population) then the equilibrium potentials for carbachol and histamine evoked responses will be the same. Let this potential be ϵ (V). A simple equivalent circuit for the membrane during activation of muscarinic or histamine receptors where the receptor operated channels produce a voltage-insensitive increment, ΔG (siemens), in the resting conductance, G (siemens), which is also insensitive to potential, gives a drug-induced depolarization of

$$\Delta V = \frac{\Delta G}{\Delta G + G}(E - \epsilon), \tag{1}$$

volts from a resting membrane potential, E (V) (Ginsborg, 1967, 1973; Bolton, 1972, 1979).

Suppose a rectangular pulse of current is applied to a smooth muscle strip in the partition chamber as used in these experiments and it evokes an electrotonic potential P (V) in size. Suppose a substance such as carbachol or histamine is applied which increases membrane conductance then the same pulse will evoke a reduced electrotonic potential P' (V) in-size. If recording is made close to the point of injection of current into an infinite cable, which is assumed to represent the smooth muscle strip, then when certain assumptions are satisfied the following approximation holds (Ohashi, 1971; Bolton, 1972):

$$\Delta V = (E-\epsilon)\{1-(P'/P)^2\} \quad (2)$$

so that

$$\epsilon = E-\frac{\Delta V}{\{1-(P'/P)^2\}} \quad (3)$$

The experiments which follow were designed to measure E, ΔV, P' and P upon application of carbachol or histamine (Table 1). A concentration of carbachol was chosen, 10^{-6} M, which previous experiments indicated would produce a depolarization similar to that produced by histamine, nominally 10^{-4} M. Experiments in which the change in size of the electrotonic potential was measured were more difficult to perform than measurements of the effect on membrane potential alone. The precaution of always using freshly prepared histamine solutions could not be observed as the experiments necessitated keeping histamine for several hours dissolved in physiological salt solution at 35 °C and pH 7·2. Under these conditions it is unstable and the real concentration of histamine at the time of application to the muscle was therefore not known. However, the experiments measure the ratio $(\Delta V)/\{1-(P'/P)^2\}$ and knowledge of the actual concentration of histamine is unnecessary. In Table 2, the average values of $(P'/P)^2$ are very different from what would be expected from the average values of P'/P; this reflects the form of the scatter of the results.

TABLE 1. Estimate of increases in conductance produced by histamine (10^{-4} M) and two concentrations of carbachol (10^{-6} M, 10^{-4} M). The change in the size of the electrotonic potential (P'/P) was used to estimate the increase in conductance $(P/P')^2$ during each response. The number of responses (n) is also given. Means ± S.E. of means.

	Histamine	Carbachol	
	10^{-4} M	10^{-6} M	10^{-4} M
Membrane potential (mV)	−44·3 ± 1·6	−42·9 ± 1·6	−40·2 ± 2·3
Drug-induced depolarization (mV)	29·5 ± 1·9	31·3 ± 1·3	31·6 ± 1·9
Average change in size of electrotonic potential (P'/P)	0·46 ± 0·04	0·33 ± 0·05	0·18 ± 0·03
Average estimate of increase in conductance $(P/P')^2$	8·3 ± 2·2	26 ± 7	174 ± 145
'Apparent' equilibrium potential calculated using equation 3 (mV)	−3·6 ± 2·9	−5·4 ± 2·0	−7·4 ± 0·9
n	(22)	(18)	(7)

Effect of histamine and carbachol on conductance

Measurements were made of the effects on conductance of roughly equi-depolarizing concentrations of histamine and carbachol (Fig. 4). Using eqn. (3) as derived above it was possible to estimate the equilibrium potentials for responses to carbachol and histamine. If the equilibrium potentials for these two stimulants are appreciably different then this should be detectable using this method. Depolarizations to histamine (nominally 10^{-4} M) recorded in twenty-two cells averaged 29·5 ± 1·9 mV. Depolarizations to carbachol (10^{-6} M) averaged 31·3 ± 1·3 mV in eighteen cells (Table 1) and in ten of these depolarizations to histamine were also recorded. The equilibrium potential calculated from eqn. (3) for histamine from measurements of the change

in size of the electrotonic potentials during these depolarizations averaged $-3{\cdot}6 \pm 2{\cdot}9$ mV ($n = 22$). The corresponding value for carbachol was $-5{\cdot}4 \pm 2{\cdot}0$ mV ($n = 18$) (Table 1). These are not significantly different. These results imply that if the channels opened by activated histamine and activated muscarinic receptors differed in their ionic selectivity to an extent which was reflected in a difference in their equilibrium potentials greater than about 8 mV, then this would have been detectable by this method.

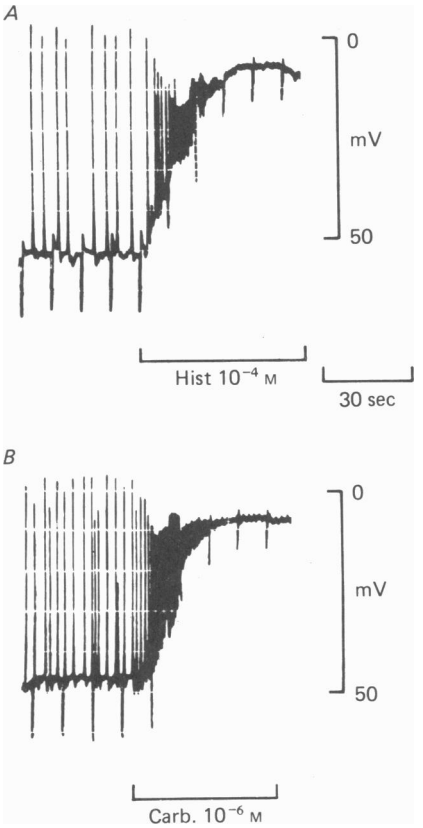

Fig. 4. Effect of histamine (Hist, 10^{-4} M) and carbachol (Carb, 10^{-6} M) on the size of the electrotonic potential elicited by 0·5 sec rectangular hyperpolarizing pulses applied every 10 sec by means of large external electrodes in the partition chamber.

Sources of error. It has been noted previously that if the depolarizing action of carbachol is insufficient to abolish action potential discharge, then accurate measurement of the change in size of the electrotonic potential is impossible (Bolton, 1972). This difficulty was encountered in these experiments with histamine. It was noticed that during depolarization some records showed an initial increase in size of the electrotonic potential before it was reduced. The initial increase occurred during the burst of increased action potential firing as the membrane potential was depolarizing. Occasionally, however, the depolarizing action of histamine was weak (presumably due to decomposition of histamine in the physiological salt solution) such that the electrotonic potential showed an anomalous increase even at the peak of the depolarizing action. Such results were discarded on the grounds that interference by potential and time-dependent membrane properties was obscuring measurement of the drug-induced increase in conductance.

Eqn. (3) upon which this comparison is based, is derived assuming that G and ΔG do not vary with potential and that channels other than drug opened channels do not change their selectivity characteristics i.e. E does not change. These assumptions are almost certainly unjustified. However, the fact that they do not hold was circumvented by eliciting equal depolarizations with carbachol and histamine. In such a case we can assume that any changes in E, ΔG, or G which might occur, do so equally in the case of the two drugs. It is, however, still possible that carbachol has effects on, for example ΔG which histamine lacks (e.g. see Bolton, 1971, 1975).

If the point of potential recording does not coincide with the point of the equivalent cable where most current crosses the membrane (i.e. $x = 0$) then the change in size of the electrotonic potential, P'/P, will over estimate the increase in conductance: thus $\{1-(P'/P)^2\} > \Delta G/\Delta G+G$. This source of error may not have been important in the present experiments as the estimate of the equilibrium potential obtained was more positive than previously suggested (Bolton, 1972, 1973). The method previously used assumed that the membrane potential in the presence of a large concentration of carbachol estimated ϵ. This is equivalent to assuming $(P'/P)^2$ is zero in eqn. (3).

The increase in conductance estimated from the ratio $(P/P')^2$ in these experiments was 26 ± 7-fold ($n = 18$) for 10^{-6} M and 174 ± 145-fold ($n = 7$) for 10^{-4} M-carbachol (Table 1). The latter value is clearly unreliable (see also Bolton, 1972) but for an increase in conductance of this magnitude $\epsilon \approx (E-\Delta V)$; i.e. the membrane potential is virtually at the equilibrium potential in the presence of this concentration of carbachol. However, this is not so in the presence of 10^{-4} M-histamine which increased conductance by an average of $8{\cdot}3 \pm 2{\cdot}2$ times ($n = 22$). This would result in a 4 mV or so difference between the membrane potential in the presence of 10^{-4} M-histamine and the equilibrium potential (eqn. (2)). The smaller maximum effect of histamine on membrane conductance explains the results described above where 10^{-4} M-carbachol depolarized about 5 mV more than 10^{-4} M-histamine. If both carbachol and histamine open ion channels with the same ionic selectivity, then carbachol's ability to open more of these channels, and thus approach their equilibrium potential more closely, explains its slightly greater maximal depolarizing action.

Effects of histamine and carbachol in combination on ^{42}K efflux

The electrophysiological experiments were consistent with the idea that the ionic selectivity of the channels opened by histamine receptor activation was similar to or the same as that of channels opened by muscarinic receptor activation. It may be that these channels represent the same population upon which both activated muscarinic or activated histamine receptors can act.

Maximum ^{42}K efflux to carbachol occurs at about 10^{-5} M (Bolton & Clark, 1981*a*, *b*) but is apparently not limited by the availability of muscarinic receptors because if these are irreversibly occluded by the use of an alkylating muscarinic antagonist then the contractile response can be larger at 10^{-4} M-carbachol than at 10^{-5} M showing that over this concentration range receptor occupancy continues to increase (Burgen & Spero, 1968; Taylor, Cuthbert & Young, 1975). We found that the application of propylbenzilylcholine mustard, an alkylating agent which irreversibly occludes muscarinic receptors (Gill & Rang, 1966; Taylor *et al.* 1975) reduced the ^{42}K efflux response to carbachol and that after this treatment the efflux response to 10^{-4} M-carbachol was significantly ($P < 0{\cdot}005$) greater than that to 10^{-5} M-carbachol (Fig. 5). If the ^{42}K efflux responses to 10^{-5} M-carbachol in the absence of propylbenzilylcholine mustard if not limited by the availability of receptors, as this result would

imply, then it may be limited by the availability of ion channels to which the activated receptors are linked.

It will be noticed in Fig. 5 that the response to 2×10^{-5} M-carbachol was substantially less in three strips upon a second application. The cause of this is not known but this effect is not the reason why, when an ascending series of carbachol

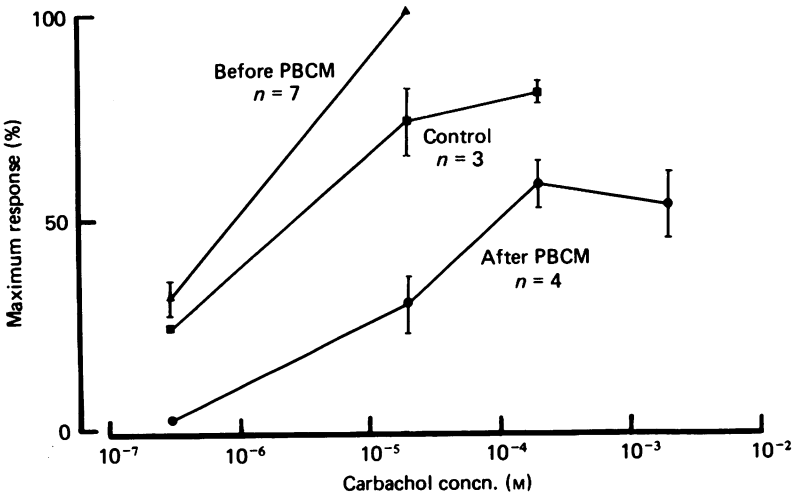

Fig. 5. Effect of irreversibly occluding muscarinic receptors with propylbenzilylcholine mustard on the ^{42}K efflux response to carbachol. Efflux responses to 3×10^{-7} and 2×10^{-5} M-carbachol were first obtained on seven ileal muscle strips. The efflux to the latter concentration was assumed to represent 100 % response. Of these strips, four were exposed to 1 or 4×10^{-7} M-propylbenzilylcholine mustard for 8 min and efflux responses to 3×10^{-7}, 2×10^{-5}, 2×10^{-4}, and 2×10^{-3} M-carbachol obtained in ascending order of concentration. The remaining strips were exposed to the same protocol except that propylbenzilylcholine mustard (PBCM) and 2×10^{-3} M-carbachol were omitted. The efflux response to 2×10^{-4} M-carbachol is significantly ($P < 0{\cdot}005$) greater than the response to 2×10^{-5} M-carbachol after treatment with the alkylating agent.

concentrations is given, the maximal efflux responses are obtained at 10^{-5} M (Bolton & Clark, 1981*a*, *b*). In a series of experiments done in conjunction with C. D. Benham, carbachol was applied at a single concentration on one occasion only, to some forty-one separate strips at either 10^{-5}, 2×10^{-5} or 10^{-4} M. The mean efflux responses are given in Table 2. They were not significantly different in size and their magnitude agreed well with values previously obtained. It is noteworthy that if concentrations *below* 10^{-5} M are applied for a brief period (say 15 sec) then the efflux response does not reach its maximum value (Bolton & Clark, 1981*a*) and this may have the effect of producing an efflux dose-response curve which will continue to increase above 10^{-5} M (Burgen & Spero, 1968).

We therefore looked at the effects of applying histamine and carbachol in combination on ^{42}K efflux in the presence of tetrodotoxin (2×10^{-7} M). The response to histamine (10^{-4} M) and carbachol (2×10^{-5} M) applied simultaneously was not greater than that with carbachol alone (Fig. 6*A*). This was not due to carbachol acting on histamine receptors at the concentration used. Mepyramine ($2{\cdot}5 \times 10^{-7}$ M, a concentration which should produce substantial blockade of histamine receptors,

TABLE 2. Increase in ^{42}K efflux rate upon application of large concentrations of carbachol as a single application to each strip 10–15 min after beginning perfusion with non-radioactive solution. The values shown are not significantly different. Experiments done in conjunction with C. D. Benham.

	Concentration of carbachol (M)		
	10^{-5}	2×10^{-5}	10^{-4}
Number of ileal strips	11	23	7
Average increase in ^{42}K efflux rate $\pm$ S.E. of mean $\times 10^2$	$9{\cdot}47 \pm 0{\cdot}92$	$10{\cdot}31 \pm 0{\cdot}63$	$9{\cdot}51 \pm 0{\cdot}44$

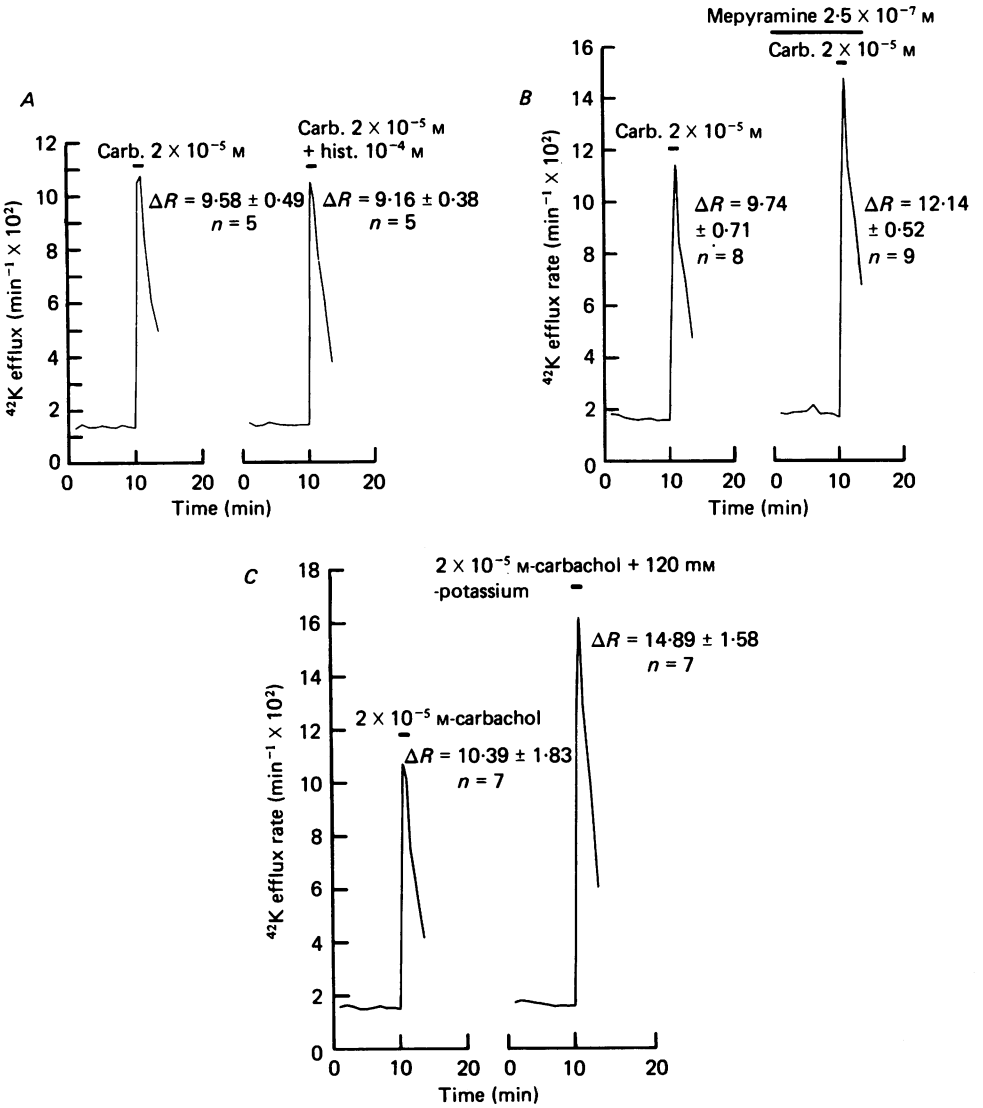

Fig. 6. *A*, effect of carbachol (2×10^{-5} M) alone and in combination with histamine (10^{-4} M, applied simultaneously) on the ^{42}K efflux rate. The lines shown are the mean responses on the indicated number of preparations (n) in each case. The mean ^{42}K efflux response (ΔR) $\pm$ S.E. of mean is given as min^{-1} multiplied by 10^2. *B*, effect of carbachol (2×10^{-5} M) alone and in the presence of mepyramine ($2{\cdot}5 \times 10^{-7}$ M) on ^{42}K efflux. Details as for *A*. *C*, effect of carbachol (2×10^{-5} M) alone and in combination with 120 mM-potassium (low sodium) solution on ^{42}K efflux response. Details as for *A*.

Schild, 1947) did not reduce the response to carbachol but increased it (Fig. 6*B*). The failure of carbachol and histamine in combination to produce a larger ^{42}K efflux response than carbachol alone was not due to some process which limits the maximum efflux of which the tissue is capable. When 120 mM-potassium solution was applied simultaneously with 2×10^{-5} M-carbachol, the efflux was $0{\cdot}149 \pm 0{\cdot}016$ min^{-1} ($n = 7$)

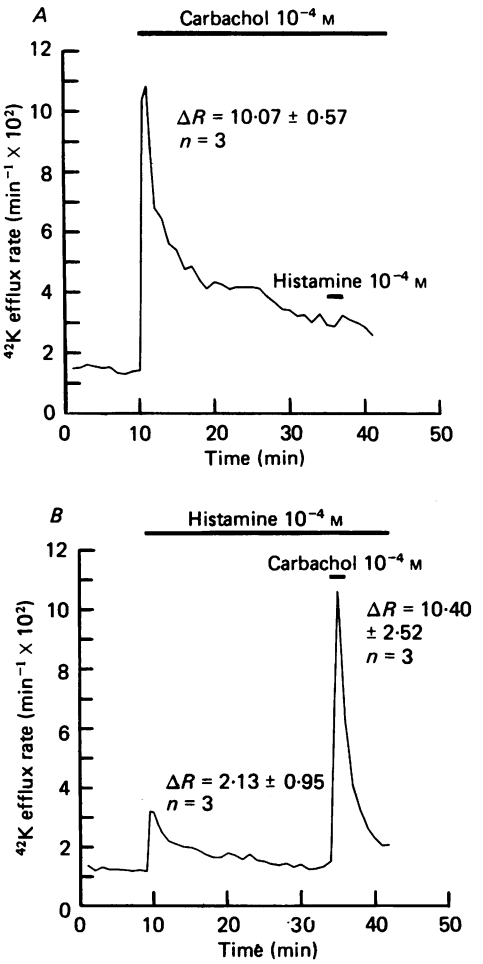

Fig. 7. *A*, effect of histamine (10^{-4} M) on the ^{42}K efflux in the presence (horizontal line) of carbachol (10^{-4} M). *B*, effect of carbachol (10^{-4} M) in the presence (horizontal line) of histamine (10^{-4} M). In both *A* and *B* the mean efflux on three preparations is shown and the ^{42}K efflux response (ΔR) is expressed as min$^{-1} \times 10^2$.

compared to $0{\cdot}104 \pm 0{\cdot}018$ min^{-1} ($n = 7$) in strips where carbachol was applied alone (Fig. 6*C*). The difference, 0·045 min^{-1}, which is significant ($P < 0{\cdot}05$ on a one tail test) is smaller than but comparable to the effect of 120 mM-potassium applied alone viz. $0{\cdot}058 \pm 0{\cdot}005$ min^{-1} (Bolton & Clark, 1981*b*). These results are consistent with the possibility that when muscarinic receptors are activated by carbachol the ion channels which are opened are from the same population as those opened by activated histamine receptors. If it is assumed that the carbachol efflux response is limited by

the number of available ion channels, then no further effect of histamine would be expected.

When carbachol or histamine were applied for periods of more than 30 min the ^{42}K efflux response declined and in the latter case efflux was barely greater than basal levels. Histamine had no effect in the presence of carbachol but carbachol had a substantial and virtually normal effect in the presence of histamine (Fig. 7). To explain the loss of the efflux response to histamine under these conditions it would be necessary to suppose that 10^{-4} M-carbachol 'uses up' all the available ion channels during the initial part of the efflux response and that they remain 'used up' even when the response declines, presumably because the process of desensitization involves channel closure. This effect does not seem to occur during the desensitization to histamine as the response to carbachol ($0{\cdot}104 \pm 0{\cdot}025$ min^{-1} $n = 3$) in the presence of histamine was larger than carbachol given alone ($0{\cdot}101 \pm 0{\cdot}057$ min^{-1} $n = 3$). If histamine had 'used up' some channels the response ought to have been smaller and detectably so.

Carbachol and histamine contractions in 120 mM-potassium solution

Contractions produced by carbachol in 120 mM-potassium solutions were often followed by a relaxation below the level of resting tone when carbachol-free solution was restored (Fig. 8*A* and *B*, see also Brading & Sneddon, 1980). Following this relaxation a second longer-lasting contraction followed which could last up to 5 min or so following larger concentrations of carbachol. Experiments showed that these triphasic tension changes were not caused by changes in temperature, pH, or oxygenation of the solution.

Histamine also produced relaxation upon returning to histamine-free solution but no, or very small, secondary contraction occurred. It seemed possible that the smaller secondary contraction with histamine may reflect its more rapid removal from within the muscle (by uptake or metabolism) upon returning to drug-free solution. However, acetylcholine also produced a secondary contraction (although generally smaller than that to carbachol) (Fig. 8*B*). Acetylcholine is presumably removed rapidly from within the muscle by the activity of cholinesterase.

Relaxations following the application of carbachol were less if the tone fell for any reason. Apart from this, no specific blocking effect on the relaxations was found with phentolamine (10^{-5} M) an α-receptor blocker, mepyramine (10^{-7} M) propranolol (10^{-6} M) a β-receptor blocker, cimetidine (10^{-4} M) an H_2-receptor blocker or by ouabain (10^{-4} M) which blocks the sodium pump. Removing the remaining 15 mM-sodium in the 120 mM-potassium solution did, however, reduce these relaxations. Not all stimulants produced these triphasic changes in length; prostaglandin E_2 produced contraction in 120 mM-potassium solution but no relaxation occurred below baseline upon returning to prostaglandin-free solution, the muscle instead slowly returning to its prestimulation length. Measurements of ^{42}K efflux in 120 mM-potassium solution were also made. Carbachol (10^{-5} M) produced an initial increase in ^{42}K efflux much smaller than in normal solution which subsided before the secondary contraction took place. It would seem that the secondary contraction may represent some intracellular event unaccompanied by changes in permeability, at least to potassium.

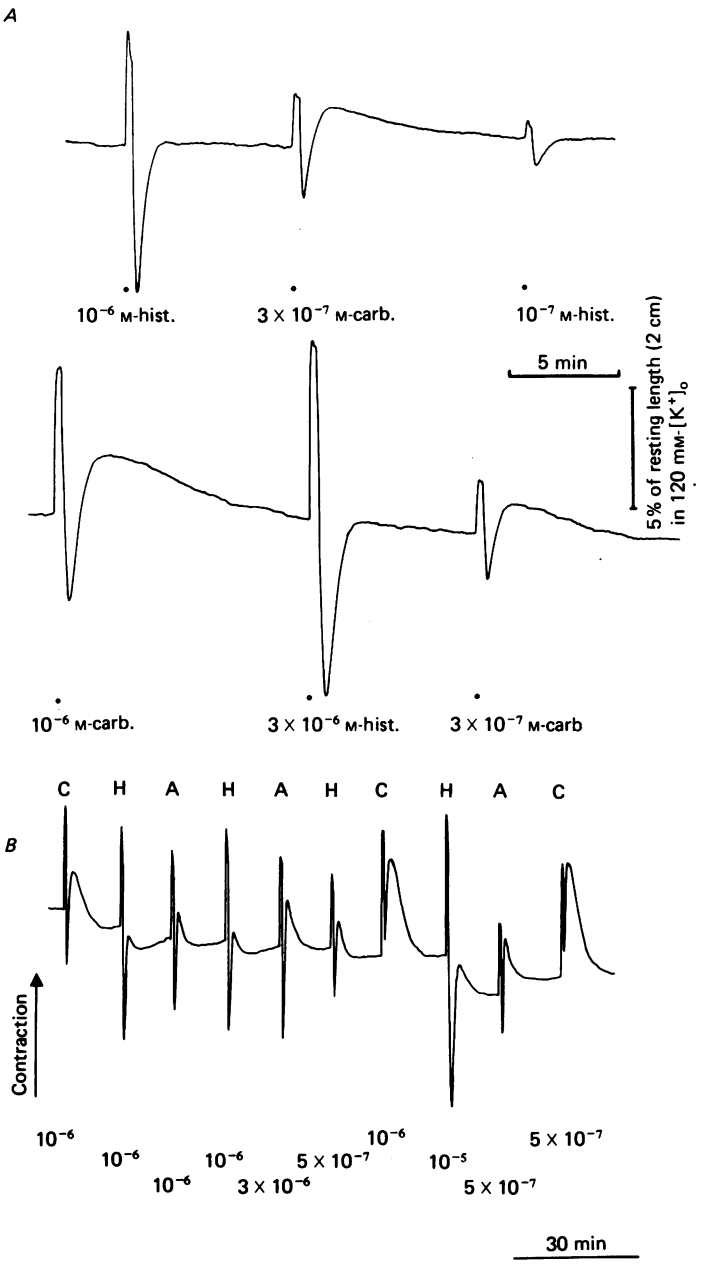

Fig. 8. *A*, changes in length of longitudinal ileal muscle in 120 mM-potassium solution to 15 sec applications of the indicated concentrations of histamine (hist.) and carbachol (carb.). *B*, as *A* but responses to 30 sec applications of carbachol (C), histamine (H) and acetylcholine (A). Notice that a relaxation below basal length follows shortening when the drug was washed from the muscle. In the case of carbachol a secondary contraction follows this relaxation. This secondary contraction is absent (*A*) or less (*B*) in the case of histamine. Acetylcholine also produced a smaller secondary contraction than carbachol. In *B* the molar concentrations of the drugs are given.

DISCUSSION

These results raise the possibility that there may be in the smooth muscle membrane a population of ion channels which can be opened by activated receptors for a number of different stimulant substances. A corollary of this would be that different types of activated receptors would differ either in their ability to open these channels or in the proportion of these channels which they were capable of opening so that maximal responses to different stimulants would not be the same. A scheme not unlike this has been implicated in the actions of stimulant substances on gland cells. In this case calcium release and/or influx has been suggested as being the primary event in the opening of common channels (Putney, 1977, 1978).

The evidence that histamine and muscarinic receptors open ion channels from the same population was in two parts: first, the identity of the reversal potentials for depolarizing responses to these two substances, and secondly the inability of a maximally effective concentration of histamine to increase ^{42}K efflux beyond that obtainable with a maximally effective concentration of carbachol.

The larger ^{42}K efflux produced by carbachol and 120 mM-potassium in combination than by carbachol alone implies that the ^{42}K efflux response to higher concentrations of carbachol is not limited by some process inherent to the muscle strip. For example, diffusion of ^{42}K out of the muscle strip or a decline of potassium concentration gradients across cell membranes could conceivably have limited the maximum ^{42}K efflux response attainable. The experiments with the receptor alkylating agent propylbenzilylcholine mustard suggested that it was not the number of muscarinic receptors which limited the ^{42}K efflux response but implied that 'spare receptors' existed. At present it would seem that the most plausible explanation is that the number of available ion channels which activated muscarinic receptors can open to allow ^{42}K to escape is the limiting factor on the ^{42}K efflux response.

This conclusion would imply that another type of receptor, for example histamine receptors, if these open the same channels, would be unable to increase ^{42}K efflux further in the presence of a maximally effective concontration of carbachol as was found. However, it must be admitted that certain features of the results are not easy to account for on this simple hypothesis. First, histamine applied in the presence of a maximally effective concentration of carbachol after the ^{42}K efflux response had declined considerably (Fig. 7*A*) failed to produce a detectable effect on ^{42}K efflux. To incorporate this observation within the scheme would necessitate an assumption that desensitized muscarinic receptors are linked in some way to closed ion channels which do not allow ^{42}K to escape and which are unavailable for opening by activated histamine receptors. The observation that mepyramine, a very specific histamine H_1 antagonist, increased the maximal ^{42}K efflux which could be elicited by carbachol is enigmatic. However, this finding does support the contention that maximum ^{42}K efflux is not limited by some property of the tissue.

The method of proving the equality of the histamine and carbachol equilibrium potentials has not previously been applied to smooth muscle. It is clear that it cannot be regarded as a method of obtaining an accurate measure of the absolute values of equilibrium potentials. Nevertheless, as a method of detecting differences between the equilibrium potentials for depolarizing responses to different substances it would

seem to be useful. It relies basically on a measurement of the relative effects of the stimulant on the membrane potential and on the size of the electrotonic potential. A slightly more elaborate formulation was used in these experiments which derives an estimate of the equilibrium potential on the basis of an assumed equivalent circuit (Ginsborg, 1967, 1973; Bolton, 1972, 1979) consisting of ohmic resistances and potential-insensitive electromotive forces. Despite these limitations, the 'equilibrium potentials' for histamine and carbachol differed by less than 2 mV, less than one standard error of the mean values. If the ionic selectivities of channels opened by histamine were grossly different from those opened by carbachol e.g. if histamine opened channels which allowed only sodium to pass, then their equilibrium potential would be expected to be substantially positive, and the driving force on the net current through the channel would be approximately double that on the net current through a channel with an equilibrium potential of about −10 mV. For the same depolarization the increase on conductance would only be half as great. Other possibilities such as histamine *reducing* potassium permeability while at the same time increasing sodium permeability seem inherently improbable in view of the effect of histamine on ^{42}K efflux. If the method is valid then differences in the equilibrium potentials of more than 8 mV ought to have been detectable given the standard errors of the measurements. Differences in equilibrium potentials of 10 mV or less must be regarded as representing rather subtle differences in selectivity of the ion channels involved. The present simple hypothesis that activated histamine and activated muscarinic receptors open ion channels with the same ionic selectivity seems plausible. It should also be testable as, providing interference at the receptor level does not occur, depolarizing responses to histamine and carbachol ought to be similarly affected by alterations of the ionic gradients across the membrane.

Histamine had a much smaller maximal effect on membrane conductance than carbachol, increasing this about 8-fold. This implies that histamine receptors open fewer ion channels when maximally activated than can be opened by maximal muscarinic receptor activation. Unfortunately we lack a selective irreversible ligand for histamine receptors which would allow experiments like those with propylbenzilylcholine mustard to be performed. Such experiments would have indicated if the number of histamine receptors was limiting on the ^{42}K efflux response. The smaller maximal increase in membrane conductance which could be obtained with histamine is consistent with the smaller maximal effect on ^{42}K efflux described here and in the previous paper (Bolton & Clark, 1981*b*) and the smaller number of histamine than muscarinic binding sites in ileal smooth muscle (Hill, Young & Marrian, 1977).

If the hypothesis is correct that common ion channels can be opened by activation of receptors for several stimulant substances then two basic types of relationship could be envisaged as existing between receptors for different stimulants and the ion channels which they open. In an 'integral-receptor model' the cell would synthesize ion channel macromolecules which might be held immobile in the smooth muscle cell membrane. Most or all of such channels would have a muscarinic receptor permanently associated with them but only a fraction (say 20 %) would have a histamine receptor. In a 'separate-receptor model' ion channels and receptors would be separate and independent entities except that, when activated, receptors would bind to the ion channels causing these to open. Muscarinic receptors in their activated state would

have a high affinity for ion channels and activated histamine receptors a lower affinity; alternatively, binding affinities may not differ but the efficacies of the bound, activated receptors to open the ion channel. The results with propylbenzilylcholine mustard imply that there are more muscarinic receptors than ion channels and this would favour a separate receptor model. A modification of the separate receptor model would invoke some enzymic process, leading to the production of a diffusible substance, which would link activated receptors to the opening of ion channels. This latter modification of the separate receptor model has certain attractive features because, notwithstanding the result obtained in the present work, the existence of a common pathway between various types of activated receptors and contraction should not be seen as precluding the existence of other pathways. Three lines of evidence support the existence of pathways specific to a particular receptor type. In the previous paper (Bolton & Clark, 1981*b*) the contractions produced by carbachol were associated with a smaller depolarization of the membrane than those produced by histamine which implies that activation of muscarinic receptors calls into play mechanisms additional to the common ion channel mechanism postulated here. The different effects of histamine and carbachol on tension in high potassium solution (Fig. 8) might also reflect the existence of such additional pathways. It has been described how contractions to some stimulants (acetylcholine) are less affected than contractions to others (prostaglandin) by anoxia, metabolic poisons and other procedures. Also the biochemical effects of various stimulants have been described as differing (see Bolton, 1979 for a review). It would seem that pathways both common to a number of receptor types for stimulants, and specific to individual receptor types, may link activated receptors to the contraction they produce in smooth muscle.

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REFERENCES

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.* **196**, 87–100.

BANERJEE, A. K. (1972). The influence of drugs upon ^{42}K fluxes in guinea-pig ileum. *Archs int. Pharmacodyn. Thér.* **198**, 173–186.

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. Pharmac.* **16**, 134–136.

BOLTON, T. B. (1971). On the nature of the oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle. *J. Physiol.* **216**, 403–418.

BOLTON, T. B. (1972). The depolarizing action of acetylcholine or carbachol in intestinal smooth muscle. *J. Physiol.* **220**, 647–671.

BOLTON, T. B. (1973). The permeability change produced by acetylcholine in smooth muscle. In *Drug Receptors*, ed. RANG, H. P., pp. 87–102. London: MacMillan.

BOLTON, T. B. (1975). Effects of stimulating the acetylcholine receptor on the current–voltage relationships of the smooth muscle membrane studied by voltage clamp of potential recorded by micro-electrode. *J. Physiol.* **250**, 175–202.

BOLTON, T. B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.* **59**, 606–718.

BOLTON, T. B. & CLARK, J. P. (1981*a*). Actions of various muscarinic agonists on membrane potential, potassium efflux, and contraction of longitudinal muscle of guinea-pig intestine. *Br. J. Pharmac.* **72**, 319–334.

BOLTON, T. B. & CLARKE, J. P. (1981*b*). Effects of histamine, high potassium and carbachol on ^{42}K efflux from longitudinal muscle of guinea-pig intestine. *J. Physiol.* **320**, 347–361.

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

BRADING, A. F. & SNEDDON, P. (1980). Evidence for multiple sources of calcium for activation of the contractile mechanism of guinea-pig taenia coli on stimulation with carbachol. *Br. J. Pharmac.* **70**, 229–240.

BÜLBRING, E. (1954). Membrane potentials of smooth muscle fibres of the taenia coli of the guinea-pig. *J. Physiol.* **125**, 302–315.

BÜLBRING, E. (1955). Correlation between membrane potential, spike discharge and tension in smooth muscle. *J. Physiol.* **128**, 200–221.

BÜLBRING, E. (1957). Changes in configuration of spontaneously discharged spike potentials from smooth muscle of the guinea-pig's taenia coli. The effect of electrotonic currents and of adrenaline, acetylcholine, and histamine. *J. Physiol.* **135**, 412–425.

BÜLBRING, E. & BURNSTOCK, G. (1960). Membrane potential changes associated with tachyphylaxis and potentiation of the response to stimulating drugs in smooth muscle. *Br. J. Pharmac. Chemother.* **15**, 611–624.

BURGEN, A. S. V. & SPERO, L. (1968). The action of acetylcholine and other drugs on the efflux of potassium and rubidium from smooth muscle of the guinea-pig intestine. *Br. J. Pharmac.* **34**, 99–115.

BURGEN, A. S. V., HILEY, C. R. & YOUNG, J. M. (1974). The binding of [^{3}H]propylbenzilylcholine mustard by longitudinal muscle strips from guinea-pig small intestine. *Br. J. Pharmac.* **50**, 145–151.

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

GILL, E. W. & RANG, H. P. (1966). An alkylating derivative of benzilylcholine with specific and long-lasting parasympatholytic activity. *Molec. Pharmacol.* **2**, 284–297.

GINSBORG, B. L. (1967). Ion movements in junctional transmission. *Pharmac. Rev.* **19**, 289–316.

GINSBORG, B. L. (1973). Electrical changes in the membrane in junctional transmission. *Biochim. biophys. Acta* **300**, 289–317.

HILL, S. J., YOUNG, J. M. & MARRIAN, D. H. (1977). Specific binding of [^{3}H]mepyramine, to histamine H_1 receptors in intestinal smooth muscle. *Nature, Lond.* **270**, 361–363.

OHASHI, H. (1971). The relative contribution of K and Cl to the total increase of membrane conductance produced by adrenaline on the smooth muscle of guinea-pig taenia coli. *J. Physiol.* **212**, 561–575.

PUTNEY, J. W. (1977). Muscarinic, α-adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland. *J. Physiol.* **268**, 139–149.

PUTNEY, J. W. (1978). Stimulus-permeability coupling; role of calcium in the receptor regulation of membrane permeability. *Pharmac. Rev.* **30**, 209–245.

SCHILD, H. O. (1947). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother.* **2**, 189–206.

TAYLOR, I. K., CUTHBERT, A. W. & YOUNG, M. (1975). Muscarinic receptors in rat intestinal muscle; comparison with the guinea pig. *Eur. J. Pharmac.* **31**, 319–326.