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Comparison of the excitatory actions of substance P, carbachol, histamine and prostaglandin $F_{2\alpha}$ on the smooth muscle of the taenia of the guinea-pig caecum

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1 A comparison was made of the actions of substance P, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), histamine and carbachol on the membrane potential and conductance of the longitudinal smooth muscle of the taenia of the guinea-pig caecum using the double sucrose gap apparatus.

2 The increases in conductance produced by the four drugs during matched depolarizations in the sucrose gap were not significantly different and they were substantially larger than the increase in conductance brought about during the same depolarization produced by passing outward current.

3 In sucrose-substituted, Na- and Cl-deficient solution the increases in conductance and depolarization due to carbachol, substance P, and $PGF_{2\alpha}$ were attenuated to a similar extent. The depolarization due to histamine under these conditions was reduced to a significantly greater extent than that due to carbachol. In Tris-benzene-sulphonate substituted Na- and Cl-deficient solution the responses due to carbachol and histamine were attenuated to a similar extent. This suggests that sucrose addition may have a specific effect on the histamine response.

4 In Tris-substituted Na-deficient solution the increases in conductance and depolarization produced by substance P, histamine and carbachol were attenuated to a similar extent. The depolarization due to $PGF_{2\alpha}$ was reduced by a significantly greater amount which may be due to unmasking an inhibitory effect that was sometimes apparent in normal solution.

5 In benzenesulphonate-substituted, Cl-deficient solution the increases in conductance and depolarizations produced by moderate concentrations of $PGF_{2\alpha}$, histamine and carbachol were attenuated to a similar extent. The response to substance P was little affected. In glucuronate-substituted, Cl-deficient solution the increases in conductance and depolarizations due to substance P and carbachol were attenuated to a similar extent. This result, and the observation that the depolarization to large concentrations of carbachol was not reduced in benzenesulphonate-substituted, Cl-deficient solution, suggest that benzenesulphonate interferes with the reactions of the non-peptide stimulants with their respective receptors.

6 The similarities in the effects of activating the four types of receptor under some conditions could be explained if they all acted on the same population of receptor-operated channels. In addition it seems that $PGF_{2\alpha}$ acts also on a population of inhibitory receptors, sucrose interferes with histamine's action, and benzenesulphonate interferes with the reactions of non-peptide stimulants with their respective receptors.

Introduction

Carbachol, substance P, histamine and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are believed to produce contraction of longitudinal smooth muscle of guinea-pig intestine by a direct action via receptors on the smooth muscle. Substance P produces a mixed direct and indirect response in ileum, although the initial component is a direct action. In the stomach it causes depolarization and bursts of action potentials (Bury & Mashford, 1977; Milenov, Nieber & Oehme, 1978; Holzer & Lembeck, 1980). Contractions of smooth muscle due to $PGF_{2\alpha}$ are also associated with increased action potential discharge and an increase in membrane conductance (Ouji, 1974; Kadlec & Radomirov, 1975; den Hertog & van den Akker, 1979; Radomirov, 1980). The actions of histamine and carbachol also appear to be directly on smooth muscle cells and in taenia this direct action depolarizes the tissue, increases membrane conductance and in-

creases potassium efflux (Bülbring & Burnstock, 1960; Durbin & Jenkinson, 1961; Paton & Zar, 1968; Bolton & Clark 1981a,b; Bolton, Clark, Kitamura & Lang, 1981; Bolton, Benham, Clark, Kitamura & Lang, 1982). Thus these four stimulants seem to have rather similar effects on tension and electrical activity.

In view of this similarity between the responses to these four stimulants we have examined the responses in a quantitative manner in an attempt to determine if a common ionic mechanism underlies them. Previous comparisons of actions of stimulants on smooth muscle have been made. Osa & Taga (1973a,b) have studied the action of carbachol and oxytocin on mouse uterus and Bülbring & Szurszewski (1974) looked at the responses of carbachol and noradrenaline in guinea-pig myometrium. In these studies no attempt was made to match the effects of the stimulants. If this is not done the contribution of voltage-dependent ion channels (Bolton, 1979) to the overall increase in conductance during drug responses will vary, which makes it difficult to compare the properties of receptor operated channels opened by activation of different receptors.

Some of these results were reported briefly at a meeting of The Physiological Society (Benham & Bolton, 1981).

Methods

Smooth muscle of the taenia of the caecum was removed after death from guinea-pigs 300–500g in body weight.

Double sucrose gap recordings

Strips of taenia muscle about 3 cm long and about 1 mm wide were cut from the apical end of the caecum. Extracellular recordings of electrical activity were made in a double sucrose gap similar to that described by Bülbring & Tomita (1969). Tension was recorded by connecting one end of the taenia strips to an isometric force transducer. This was constructed as described by Eisner & Lederer (1979) from a strain gauge element (AE 803, Aksjeselskapet Microelectronik, Holten, Norway). The rate of flow of physiological salt solution (PSS) over the node of active muscle was kept constant at about 3 ml min^{-1}. Drugs were applied by slow injection in a small volume from two syringes by means of an infusion pump (Harvard apparatus). Bathing solutions could be changed by switching reservoirs and drugs were sometimes applied by this means. The effects of stimulant agents on membrane conductance were estimated from the change in size of the hyperpolarizing electrotonic potential. If the resting conductance is G (Siemens) and the increment in conductance produced by the stimulant agent ΔG, then we assume that $P \propto 1/G$ and $P' \propto 1/(\Delta G + G)$ where P and P′ are the sizes of the hyperpolarizing potential before and in the presence of the stimulant agent when conditions were steady. Thus, the percentage increase in conductance produced by the stimulant agent will be $(\Delta G/G) \times 100 = (P/P' - 1) \times 100$. This value is shown in Tables 1–5. In solutions of changed ionic composition, a similar treatment was used; the percentage increase in conductance obtained under these conditions $(\Delta G'/G') \times 100$ was subtracted from the percentage increase in conductance observed in same tissue in normal solution, $(\Delta G/G) \times 100$, and the value obtained is shown in the last columns of Tables 3–5, as a reduction in the percentage increase in conductance produced by the stimulant drug.

It was undesirable to elicit large electrotonic potentials as the current-voltage relationship may not be linear for large displacements from the resting membrane potential. However, if only small electrotonic potentials were elicited, accurate measurement of their change in size is impossible. Electrotonic potentials of 10–15 mV in size were normally elicited and, except where indicated, concentrations of drugs applied were chosen so that the ratio $\Delta G/G$ (or $\Delta G'/G'$) lay between 0.5 and 1.0. Larger increases in this ratio result in very small electrotonic potentials in the presence of the stimulant, which results in potentially large inaccuracies in the estimated $\Delta G/G$ ratio.

Solutions and drugs

The physiological saline solution had the following composition (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5 and glucose 11. The solution was gassed with 95% O_2: 5% CO_2 and its pH was about 7.2.

In the low sodium and low chloride solution, all sodium chloride was replaced with sucrose to give a solution containing 17 mM Na and 13 mM Cl. For sodium-deficient solutions, all the sodium chloride was replaced with Tris Cl (hydroxymethylaminomethane, Sigma). For chloride-deficient solutions, sodium and potassium chloride were replaced by sodium and potassium benzenesulphonate (BDH). In some experiments, sodium glucuronate (Koch-Light Labs.) was used as the substitute. Low sodium and low chloride solution was also made up using Tris benzenesulphonate prepared by tritating Tris with benzenesulphonic acid (BDH) to pH 7.2.

The following drugs were used: carbachol chloride (BDH); atropine sulphate (Sigma); histamine acid phosphate (BDH); prostaglandin $F_{2\alpha}$ (Upjohn); substance P (Sigma). All other salts were Analar grade.

Statistical analysis

Significance was tested by Student's *t* test (paired or unpaired where appropriate). Differences were considered significant if $P < 0.05$.

Results

The taenia of guinea-pig caecum was electrically quiescent in the double sucrose gap apparatus. Action potential discharge and contraction could be evoked by applying rectangular depolarizing current pulses (2.5 s duration). These were alternated with rectangular hyperpolarizing current pulses and the steady-state size of the resulting electrotonic potentials was used to estimate membrane conductance. Recordings were not attempted for at least 1 h after introducing the muscle into the apparatus as drug-sensitivity increased and the electrical threshold for evoking action potentials decreased during this time.

Responses in normal ionic environment

Carbachol, substance P, histamine and $PGF_{2\alpha}$ all caused depolarization, an increase in membrane conductance (as indicated by a decrease in the size of the electrotonic potentials) and contraction of the muscle. Further contraction was evoked during depolarizing current pulses. Responses to carbachol were blocked by atropine (5×10^{-8} M) but responses to the other three drugs were unaffected by atropine, indicating that their actions were not mediated indirectly by release of acetylcholine from nerve endings associated with the muscle (cf. Paton & Zar, 1968).

Table 1 Percentage increase in conductance for matched depolarizations by pairs of drugs

Drug	*Depolarization* (mV)	*% increase in conductance* ($\Delta G/G \times 100$)
Carbachol	12.0 ± 0.7	79 ± 10
Substance P	12.2 ± 0.7	77 ± 13
$n = 18$		
		Difference 3 ± 6
		NS
Carbachol	12.4 ± 0.8	79 ± 11
Histamine	12.6 ± 0.7	74 ± 10
$n = 15$		Difference 5 ± 8
		NS
$PGF_{2\alpha}$	11.6 ± 0.7	53 ± 03
Carbachol	11.7 ± 0.6	48 ± 07
$n = 12$		
		Difference 5 ± 6
		NS
Histamine	11.3 ± 0.7	61 ± 12
$PGF_{2\alpha}$	11.3 ± 0.6	54 ± 08
$n = 10$		
		Difference 7 ± 8
		NS

Significance of differences was tested by a paired two-tailed *t* test, NS indicates $P > 0.05$.

Comparisons were made between two stimulants in any one experiment. The concentration of one stimulant was varied until it produced a depolarization with its associated percentage increase in membrane conductance of 50 to 100%, as this was the range of response that could be most accurately measured. Concentrations of the second drug were tried until a matching depolarization was obtained and then the match was confirmed by repeating the appropriate concentration of the first drug. The two matched depolarizations to the two stimulants and their associated percentage increases in conductance could then be measured. The concentrations of drugs used varied from preparation to preparation and were in the range, carbachol $1-4 \times 10^{-7}$ M, histamine $10^{-5}-10^{-4}$ M, substance P $2-5 \times 10^{-6}$ M, $PGF_{2\alpha}$ $2-5 \times 10^{-5}$ M. Drugs were applied for sufficient time for a steady level of depolarization to be reached (Figures 1–6).

The results of a number of experiments of this type are summarised in Table 1. In each of four series of experiments two drugs were compared as described. As the depolarizations in individual experiments were closely matched, the averaged paired depolarizations in the four series differed by only 0.1–0.2 mV. The percentage increases in conductance produced by the four stimulants were not significantly different, the differences being smaller than the standard errors in all cases.

However, although the responses were essentially similar some differences were apparent. On some occasions $PGF_{2\alpha}$ showed less tendency to generate action potentials and, when this occurred, tension development was less and often showed an initial inhibitory phase (Figure 1). Measurements of tension generated during the matched depolarizations shown in Table 1 indicated that carbachol, substance P and histamine produced similar generation of tension. However $PGF_{2\alpha}$ was significantly less effective at stimulating contraction (Figures 1 and 5). It may be that $PGF_{2\alpha}$ activates both inhibitory and excitatory receptors in this muscle. Stimulant actions due to substance P, carbachol and histamine started immediately on application and subsided as depolarization declined (Figures 1–6). The depolarization due to $PGF_{2\alpha}$ declined more slowly following application and this resulted in a prolonged stimulant phase (Figure 1). These differences in time course of action probably reflect differences in the way the stimulants reach, or are removed from, their sites of action.

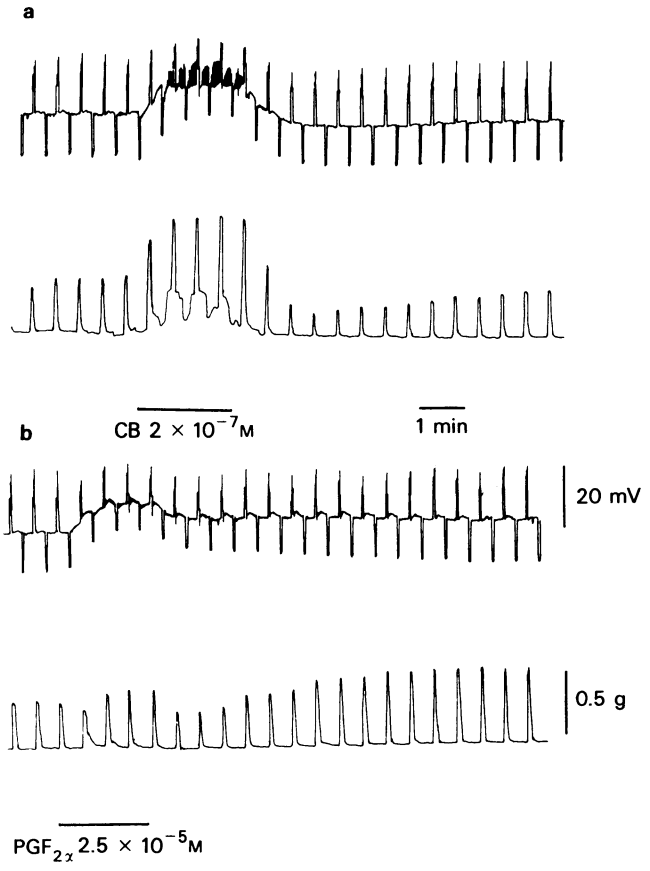

Figure 1 Effect of (**a**) carbachol 2×10^{-7}M and (**b**) $PGF_{2\alpha}$ 2.5×10^{-5}M, on membrane potential, membrane conductance and tension of taenia smooth muscle recorded in double sucrose gap. Electrical potential changes evoked by constant current pulses (2.5 s duration, every 15 s, alternately depolarizing and hyperpolarizing). Traces (a) and (b) are continuous. Horizontal line indicates period of drug application. In each panel, top tracing, electrical potential, bottom tracing, muscle tension. Note for matched drug-induced depolarizations the decrease in size of the hyperpolarizing electrotonic potentials is similar.

Comparisons with electrical and high potassium depolarizations

In order to assess the contribution of potential-sensitive ion channels to the drug-induced increase in conductance, depolarizations produced by passing outward current were compared with those produced by the stimulants. Stepwise depolarizations of 30 s duration were evoked by passing outward current of increasing strength in successive steps. During each step a 2.5 s hyperpolarizing pulse was applied to estimate membrane conductance. Following the series of depolarizations a drug was applied and the effects compared. The percentage increase in conductance for an electrically induced depolarization of the same size as that produced by the drug was obtained by interpolation. Three series of experiments were carried out in which carbachol, $PGF_{2\alpha}$ and histamine-induced depolarizations were compared with electrical depolarization (Table 2). For matched electrically induced depolarization the percentage increase in conductance was always considerably less. This indicates that the depolarization induced by the drugs was not solely responsible for the increase in conductance observed. The additional increase in conductance seen with the drug-induced responses is presumably due to opening of receptor-operated ion channels.

Histamine-induced depolarizations were compared to those produced by raising external potassium to between 35 and 45 mM in the presence of atropine (5×10^{-8}M). High-K caused a much larger fractional increment in conductance than a matched histamine depolarization in the same muscle strip

Table 2 Percentage increase in conductance for matched depolarizations produced by stimulants and outward current

Stimulant	*Number of experiments*	*Depolarization* (mV)	*% increase in conductance* ($\Delta G/G) \times 100$
Carbachol	6	12.8 ± 0.5	79
Outward current			25
			Difference 54 ± 13
			$P < 0.025$
Histamine	5	12.2 ± 0.8	67
Outward Current			14
			Difference 53 ± 15
			$P < 0.05$
$PGF_{2\alpha}$	5	11.5 ± 1.0	52
Outward current			16
			Difference 36 ± 5
			$P < 0.005$
Histamine	9	12.0 ± 4.0	45
High $[K^+]_o$			162
			Difference 117 ± 44
			$P < 0.05$

Table 3 Effects of sodium and chloride deficient (17 mM, 13 mM, sucrose substituted) solution on matched depolarizations and their associated increases in conductance produced by four different stimulants

	Normal solution		*Effects of Na and Cl deficient solution*	
	Depolarization (mV)	*% Increase in conductance* ($\Delta G/G) \times 100$	*Reduction in Depolarization* (mV)	*Reduction in effect on conductance* (%)
Carbachol	9.5 ± 1.0	78 ± 15	4.6 ± 0.4	65 ± 16
Substance P	10.2 ± 1.0	81 ± 27	5.4 ± 0.9	73 ± 25
$n = 5$				
			Difference 0.8 ± 0.9	Difference 8 ± 11
			NS	NS
Carbachol	13.7 ± 0.9	103 ± 20	4.6 ± 1.7	60 ± 24
Histamine	13.8 ± 0.8	96 ± 22	7.5 ± 1.5	91 ± 21
$n = 5$				
			Difference 2.9 ± 0.6	Difference 31 ± 12
			$P < 0.05$	NS
Carbachol	12.2 ± 1.1	45 ± 15	4.6 ± 1.3	24 ± 11
$PGF_{2\alpha}$	11.8 ± 1.3	55 ± 8	6.6 ± 1.1	39 ± 6
$n = 5$				
			Difference 2.0 ± 1.1	Difference 16 ± 9
			NS	NS
Histamine	13.3 ± 1.2	66 ± 16	9.4 ± 1.1*	63 ± 15*
Carbachol	13.6 ± 1.3	73 ± 19	10.3 ± 1.2*	70 ± 20*
$n = 5$				
			Difference 0.9 ± 0.4	Difference 5 ± 7
			NS	NS

Stimulants were applied 14 and 21 min after changing to ion deficient solution. For further details see text.
*Tris benzenesulphonate was used as the substitute.

(Table 2). This result is perhaps surprising but consistent with the observations of Bolton & Clark (1981b) who measured increases in ^{42}K efflux in ileal longitudinal muscle and found that high-K produced a larger increase in efflux than an equidepolarizing concentration of histamine.

Responses in altered ionic environment

After first eliciting matched depolarizations in normal solution, responses were then obtained in solutions of altered ionic composition to see if the responses were similarly affected. If the mechanism of action of the four stimulants was to open ion channels with the same ionic selectivity then altering the ionic gradients across the cell membrane should have similar effects on the responses.

Sodium and chloride-deficient solution

Experiments were performed in which sodium and chloride were reduced to 17 mM and 13 mM respectively by replacing sodium chloride with sucrose. Responses to carbachol, substance P and $PGF_{2\alpha}$ were rather similarly affected by sucrose replacement; depolarization was reduced by about a half in each case. The percentage increase in conductance was also reduced by a similar amount in these two sets of experiments (Table 3). when responses to histamine were compared with those elicited by carbachol, the depolarization due to histamine was reduced more than that due to carbachol and the percentage increase in conductance was also reduced by more (Figure 2), but not significantly (Table 3).

In an attempt to discover if this differential action of the ionic modification was due to the reduction in sodium and chloride concentrations or to a specific effect of the addition of sucrose, a further series of experiments were performed in which sodium and chloride were reduced to 17 and 13 mM using Tris-benzenesulphonate as the substitute. In this series of experiments the responses to histamine and carbachol application were affected to a similar extent. Depolarization was reduced by about 75% and the percentage increase in conductance largely abolished for both drugs (Figure 3 and Table 3). A possible explanation of the different effects of sucrose is that it has a specific effect on the histamine drug-receptor interaction which may be due to the large reduction in ionic strength of the bathing solution when sucrose is used as an ion substitute.

Sodium-deficient solutions

Two series of experiments were performed in which sodium was reduced to 17 mM by replacement of sodium chloride with Tris chloride. Depolarizations to carbachol, substance P and histamine were all reduced by about two thirds (Figure 4) (Table 4). The percentage increase in conductance was also reduced in each case. However depolarizations due to $PGF_{2\alpha}$ were almost completely abolished and in three experiments hyperpolarization was observed in this ionic environment (Figure 5). The percentage increase in

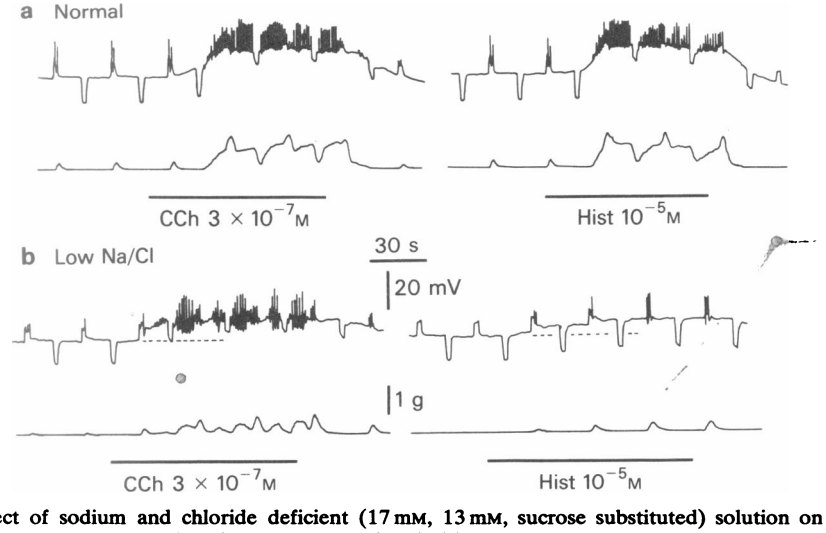

Figure 2 Effect of sodium and chloride deficient (17 mM, 13 mM, sucrose substituted) solution on matched excitatory responses to carbachol (CCh) and histamine (Hist). (a) Matched depolarizations in normal solution; (b) the effect of the same drug concentrations applied after 14 and 21 min in sodium- and chloride-deficient solution. Dotted line represents resting membrane potential before drug application.

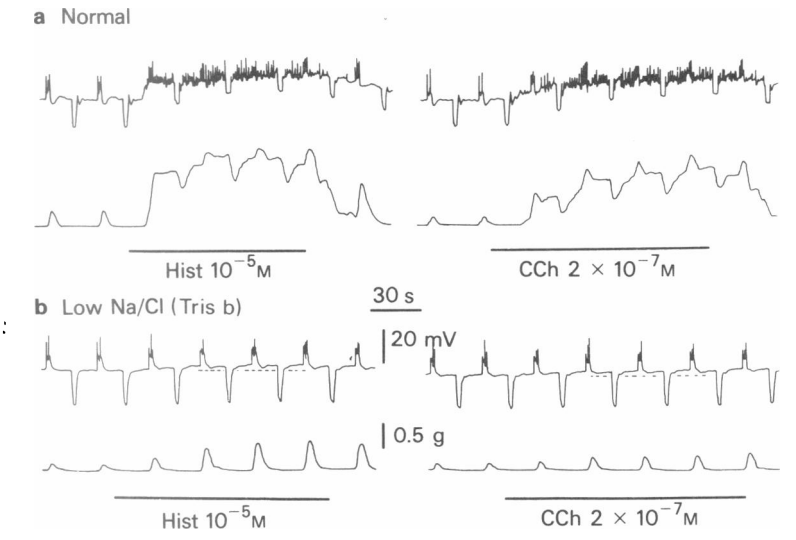

Figure 3 Effect of sodium- and chloride-deficient (17 mM, 13 mM, Tris benzenesulphonate substituted) solution on matched excitatory responses to histamine (Hist) and carbachol (CCh). (a) Matched depolarizations in normal solution; (b) the effect of the same drug concentrations applied after 14 and 21 min in sodium- and chloride-deficient solution.

conductance to $PGF_{2\alpha}$ was less affected by low sodium than that due to histamine. In all cases ($n = 6$) application of $PGF_{2\alpha}$ in low sodium inhibited contractions evoked by depolarizing electrotonic potentials (Figure 5). Thus, while substance P, histamine and carbachol were similarly affected, $PGF_{2\alpha}$ responses were affected differently by low sodium solution. Responses to $PGF_{2\alpha}$ in normal solution indicated that it might activate both inhibitory and excitatory receptors. Reduction of the excitatory response in low sodium might then unmask the response due to stimulation of inhibitory receptors.

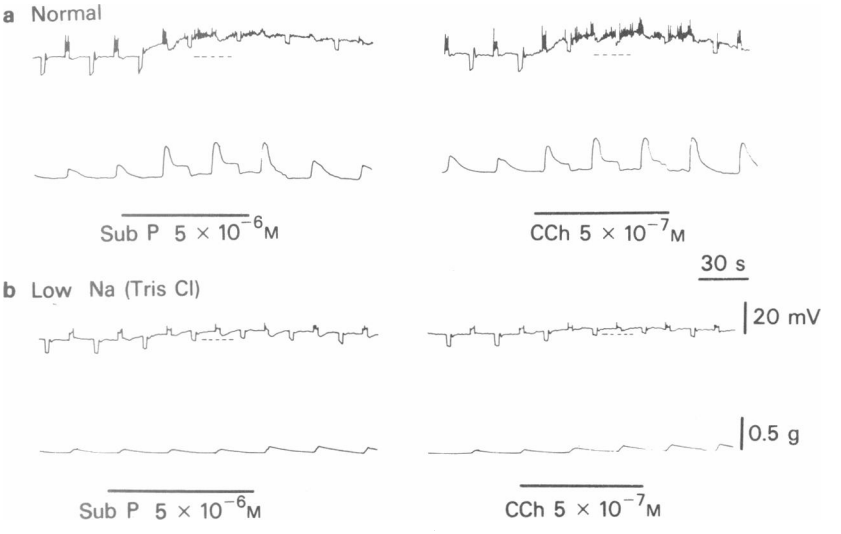

Figure 4 Effect of sodium-deficient (17 mM, Tris substituted) solution on matched excitatory responses to substance P (Sub P) and carbachol (CCh). (a) Matched depolarizations in normal solution; (b) the effect of the same drug concentrations applied after 10 and 16 min in sodium-deficient solution. Note similar reductions in depolarization and contractile response.

Table 4 Effects of sodium-deficient (17 mM, Tris substituted) solution on matched depolarizations and their associated increases in conductance produced by four different stimulants

	Normal solution		*Effects of Na-deficient solution*	
Drug	*Depolarization* (mV)	*% increase in conductance* ($\Delta G/G$) $\times 100$	*Reduction in depolarization* (mV)	*Reduction in effect on conductance* (%)
$PGF_{2\alpha}$	12.2 ± 1.1	54 ± 12	11.2 ± 0.8	13 ± 17
Histamine	11.3 ± 0.8	69 ± 19	7.7 ± 1.1	54 ± 19
$n = 6$				
			Difference 3.5 ± 0.8 $P < 0.025$	Difference 41 ± 9 $P < 0.01$
Carbachol	12.6 ± 0.8	123 ± 16	8.2 ± 1.0	30 ± 18
Substance P	12.1 ± 0.6	94 ± 31	8.3 ± 0.7	29 ± 9
$n = 5$				
			Difference 0.1 ± 0.8 NS	Difference 1 ± 21 NS

Stimulants were applied 10 and 16 min after changing to sodium-deficient solution at which time the effects on membrane potential and conductance due to changing to that solution had become steady. Differences in Na-deficient solution are expressed as described in the text for Table 3.

Chloride-deficient solutions

When the chloride concentration was reduced to 7 mM by replacing sodium and potassium chloride with sodium and potassium benzenesulphonate, both the depolarization and the percentage increase in conductance produced by the four stimulants were attenuated. When carbachol responses were compared to those to histamine and to $PGF_{2\alpha}$, the reduction in depolarization was similar, about one third and two thirds in the two series of experiments (Table 5). The reduction in the percentage increase of conductance was also similar in each series of experiments. However, when carbachol responses were compared to those of substance P it was found that depolarization due to substance P was hardly affected and the percentage increase in conductance was also reduced by a significantly smaller amount when compared to the carbachol responses (Figure 6, Table 5).

The reduction of the response to carbachol in low chloride was unexpected because in ileum (Bolton 1972; 1973) and in uterus (Bülbring & Szurszewski,

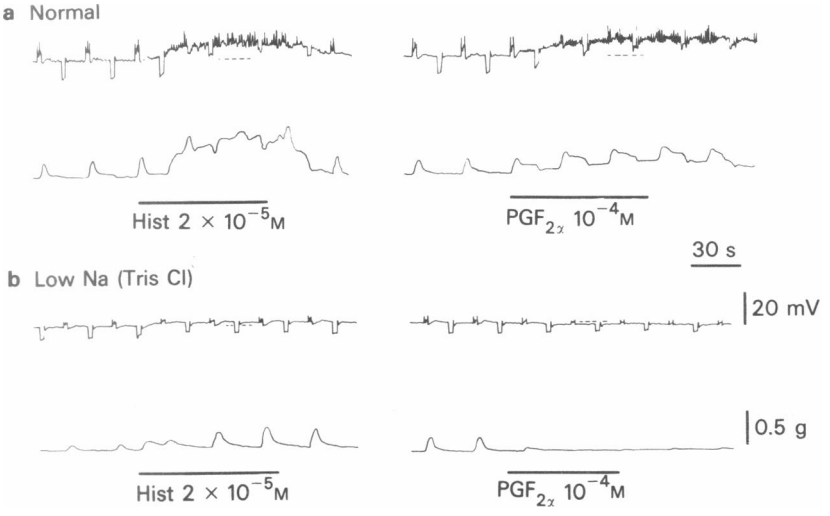

Figure 5 Effect of sodium-deficient (17 mM, Tris-substituted) solution on matched excitatory responses to histamine (Hist) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). (a) Matched depolarization in normal solution, (b) the effect of the same drug concentrations applied after 10 and 16 min in sodium-deficient solution. Note hyperpolarization and inhibition of contraction on $PGF_{2\alpha}$ application in sodium-deficient solution.

Table 5 Effects of chloride-deficient (7 mM, benzenesulphonate substituted) solution on matched depolarizations and their associated increased in conductance produced by four different stimulants

	Normal solution		*Effects of Cl-deficient solution*	
Drug	*Depolarization* (mV)	*% increase in conductance* $(\Delta G/G) \times 100$	*reduction in depolarization* (mV)	*Reduction in effect on conductance* (%)
Carbachol	9.7 ± 0.9	67 ± 11	6.1 ± 0.4	44 ± 9
$PGF_{2\alpha}$	9.7 ± 0.8	52 ± 10	5.5 ± 0.8	36 ± 13
$n = 5$				
			Difference 0.6 ± 0.9 NS	Difference 8 ± 13 NS
Carbachol	11.9 ± 0.7	81 ± 21	5.1 ± 0.7	61 ± 19
Histamine	12.4 ± 0.5	80 ± 19	4.4 ± 0.9	60 ± 16
$n = 5$				
			Difference 0.7 ± 1.2 NS	Difference 1 ± 14 NS
Carbachol	13.6 ± 1.6	99 ± 18	9.7 ± 1.7	91 ± 16
Substance P	12.2 ± 1.0	67 ± 20	0.9 ± 1.2	38 ± 17
$n = 6$				
			Difference 8.8 ± 2.0 $P < 0.01$	Difference 53 ± 13 $P < 0.025$
Carbachol	8.7 ± 1.4	68 ± 17	4.0 ± 1.6*	47 ± 19*
Substance P	8.4 ± 1.3	58 ± 23	2.6 ± 1.3*	28 ± 14*
$n = 5$				
			Difference 1.4 ± 0.7 NS	Difference 19 ± 17 NS
			Increase in depolarization (mV) †	
Carbachol 2×10^{-5} M	21.8 ± 1.6	—	11.0 ± 2.9 $P < 0.05$	—
$n = 4$				

Stimulants were applied 14 and 21 min after changing to chloride-deficient solution at which time the effects on membrane potential and conductance due to changing to that solution had become steady. Differences in Cl-deficient solution are expressed as described in the text for Table 3.

* Effects of chloride deficient (7 mM, glucuronate substituted) solution.

Conductance increases were too large to be measured accurately.

† In Cl-deficient benzene sulphonate substituted solution the response was increased and this increase was significant ($P < 0.05$).

1974) no reduction of the response to a maximally effective depolarizing concentration of carbachol was found. We repeated our experiments with a higher dose of carbachol (2×10^{-5} M), as used by these authors, rather than $1-4 \times 10^{-7}$ M. With this higher concentration, the depolarization was 21.8 mV ± 1.6 mV in normal solution and was significantly greater, 32.8 ± 2.9 mV, in low chloride solution (Table 5). Thus the effects of benzenesulphonate are different at the two carbachol concentrations. If the effects of the ion replacement were due to an increase in the chloride electrochemical gradient then responses to low and high concentrations of carbachol would be expected to be affected in a similar way. As this was not the case the explanation of the discrepancy may be that benzenesulphonate ions interfere with drug-receptors binding. With high concentrations of carbachol the level of depolarization will be rather insensitive to moderate changes in receptor occupation because of the hyperbolic relationship between the increment in conductance and depolarization (Bolton, 1979). This will not be true with modest increases in conductance.

In a further series of experiments, glucuronate was used as a chloride substitute and responses to substance P and carbachol were both reduced in low chloride; carbachol responses were still reduced by more but not significantly so (Table 5).

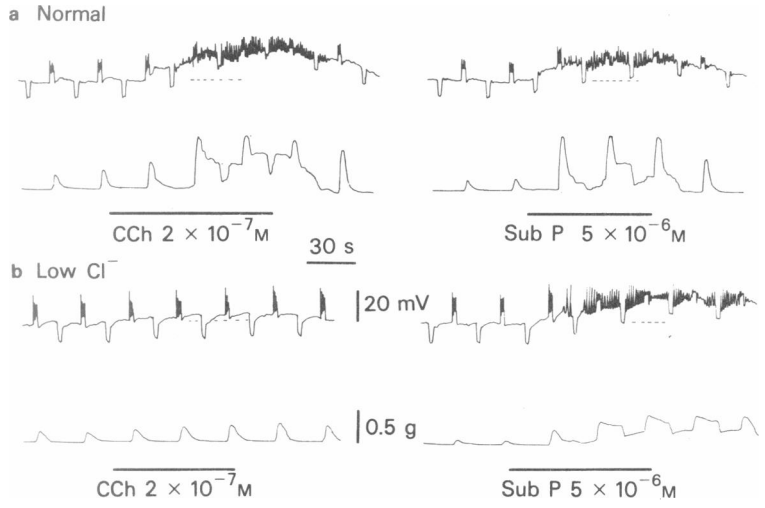

Figure 6 Effect of chloride-deficient (7 mM, benzenesulphonate substituted) solution on matched excitatory responses to carbachol (CCh) and substance P (Sub P). (a) Matched depolarization in normal solution; (b) the effect of the same drug concentrations applied after 14 and 21 min in chloride-deficient solution.

Discussion

Excitatory responses in smooth muscle that are dependent upon membrane depolarization are thought to be mediated through the opening of several types of ion channel, e.g. receptor-operated channels, voltage-sensitive channels (potassium, sodium/calcium) and calcium-activated channels (Bolton, 1979). We have shown that for matched depolarizations these four drugs cause a larger increase in conductance than that due to electrical depolarization alone, suggesting that there are indeed receptor-operated channels in addition to purely voltage-gated channels.

When drug-induced responses were compared to matched depolarizations due to high external potassium it was found that high K^+ caused a greater increase in conductance. Single channel recording techniques have shown that elevating potassium levels increases the unit conductance of individual K^+ channels (Pallotta, Magleby & Barrett, 1981). Thus depolarization produced by raising $[K^+]_o$ would be expected to have a greater effect on membrane conductance than that produced by passing outward current across the membrane.

As the matched depolarizations evoked by the four drugs produced similar increases in conductance, this suggests that the receptor-operated channels opened by them may have similar ionic selectivities. However, in each of the ion substitutions, observations were made that seemed to be inconsistent with this hypothesis. Changing the ionic gradients should affect all the responses equally if similar ion channels are involved (see Ginsborg, 1967). When making ion substitutions and measuring the effects mediated by drug-receptor interactions, the ionic environment of the receptors is being changed as well as the ionic gradient across the cell membranes. It is feasible that certain foreign ions may selectively interfere either with binding of agonists at a particular receptor or with the specific mechanism by which that activated receptor is linked to the ionic channel.

In sodium- and chloride-deficient solution, the response to histamine is reduced more than the carbachol response when sucrose is the substitute. However, when Tris benzenesulphonate was the substitute, the responses were similar and also when the Na^+ and Cl^- were reduced separately the responses to the two drugs were similarly reduced. Thus the result with sucrose is inconsistent with the other ionic changes, which suggests sucrose may be having a specific effect on the histamine response at the drug-receptor level.

When benzenesulphonate was used as a substitute for chloride, the responses to substance P were much less affected than those of carbachol, histamine or $PGF_{2\alpha}$. However, no difference between substance P, carbachol and $PGF_{2\alpha}$ was seen with sucrose substitution when chloride is also reduced. When a high concentration of carbachol was used, the depolarization was increased in low chloride solution (the increase probably results from membrane hyperpolarization which occurred in low chloride, cf. Bolton, 1972). These last two observations are not consistent with the shift in the chloride equilibrium potential affecting the carbachol response. They can be exp-

lained if benzenesulphonate ions affected carbachol binding to muscarinic receptors. Inhibition of binding of 5×10^{-7} M carbachol to muscarinic receptors by isethionate has been reported (Birdsall, Burgen, Hulme & Wells, 1979) although isethionate did not affect binding of 2×10^{-5} M carbachol. Reduction of the external chloride concentration by substitution with glucuronate produced similar effects on the carbachol and substance P responses. These results imply that benzenesulphonate ions interfere with the reactions of non-peptide stimulants with their respective receptors.

The only drug that consistently showed different responses was $PGF_{2\alpha}$. The $PGF_{2\alpha}$ depolarization was attenuated much more than the other drug responses in low sodium (Tris substituted) solution, and the same effect was seen to a lesser extent when sucrose was the substitute although the differences were not significant here. The inhibitory component of the $PGF_{2\alpha}$ response was also apparent at times in normal solution. These results could be explained by the action of $PGF_{2\alpha}$ at both excitatory and inhibitory receptors as has been observed in cat tracheal smooth muscle (Apperley, Coleman, Kennedy & Levy, 1979).

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