Br. J. Pharmac. (1970), **40**, 492–500.

The effects of calcium and magnesium on the response of intestinal smooth muscle to drugs

A. S. V. BURGEN AND L. SPERO*

Department of Pharmacology, University of Cambridge

Summary

1. The sensitivity of the longitudinal muscle of the guinea-pig ileum to muscarinic drugs producing contraction depends on optimum concentrations of calcium and magnesium. It can also be reduced by changes in sodium concentration and osmolarity.

2. The rubidium efflux response to these same drugs is insensitive to any of these changes in the external medium.

3. Raised calcium or magnesium concentration has the effect of largely annulling the differences in structure-activity relationships of the two responses as they exist in optimal media.

4. The effects are explained in terms of a labile coupling process between a single receptor and the contractile process compared with a stable coupling process of the efflux process.

Introduction

In a previous paper (Burgen & Spero, 1968) we have shown that a comparison of the effects of muscarinic drugs on the efflux of K or Rb and on the contraction in smooth muscle showed some striking quantitative differences which were difficult to reconcile with the notion of a single type of muscarinic receptor. During the course of this work we noticed that while the rubidium efflux response was insensitive to changes in calcium ion concentration very dramatic changes in the contractile response occurred with quite small changes in calcium concentration. This further divergence in character of the two responses has now been studied in some detail.

Methods

The measurements of the efflux of ^{86}Rb from strips of longitudinal muscle from ileum and the taenia coli of the guinea-pig were made as described previously (Burgen & Spero, 1968). The basic salt solution used was a Krebs bicarbonate solution of the following composition: Na, 143 mM ; K, 5·5 mM ; Ca, 2·5 mM ; Mg 2·4 mM ; Cl, 139 mM ; HCO_3, 25 mM ; glucose, 11 mM. Changes in Ca or Mg were made by changing the amount of $CaCl_2$ or $MgCl_2$ without changes in other constituents of the solution so that they involved a small change in chloride concentration and

* Present address: Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada.

in osmolarity. In low sodium solution sodium chloride was replaced by equi-osmolar amounts of sucrose.

f is the efflux rate constant expressed as min^{-1}. Apparent affinity constant K_f and the maximum efflux, f_{max}, were obtained by statistical fitting of a mass equation to the experimental points as described previously. An apparent affinity constant K_c and maximum were obtained on the contraction data in the same way. The contraction maximum was normalized to 100 in the reference experiment in each series.

Results

Influence of calcium on the efflux and contractile responses of the longitudinal muscle of the ileum

In Fig. 1 are shown the results of a typical experiment in which the dose-response relationships for the efflux and contractile responses in response to carbachol were

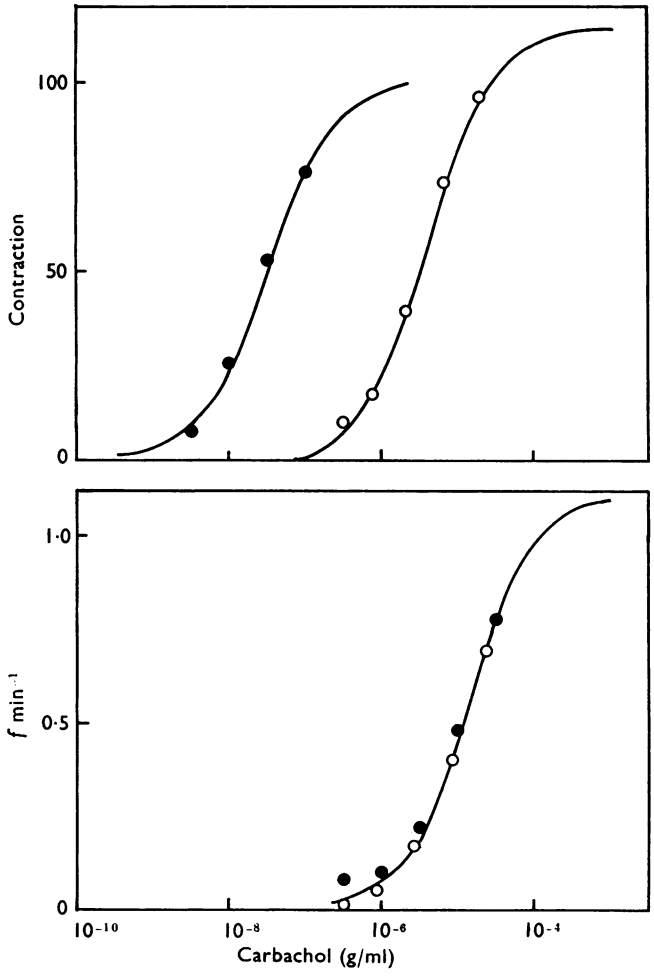

FIG. 1. Dose response curves for the contractile response (upper section) and flux response (lower section) in response to carbachol in 2·5 mM Ca^{2+} (●——●) and 5·0 mM Ca^{2+} (○——○). Abscissa: carbachol concentration g/ml. Ordinates: concentration % of maximum in 2·5 mM Ca: efflux rate constant (f min^{-1}).

measured first in Krebs solution containing the usual amount of calcium 2·5 mM (magnesium 2·4 mM), and then, in Krebs solution with the calcium concentration doubled, 5·0 mM (magnesium maintained at 2·4 mM). It can be seen that the efflux response was practically unaffected by this change in calcium concentration but the sensitivity of the contractile response was dramatically reduced by a factor of about 100. The maximum contractile response was modestly increased. To our surprise a curiously similar change occurred also when the calcium concentration was reduced to 1·25 mM (Fig. 2). A study of a range of calcium concentrations from 1·25 to 10 mM showed that calcium was without influence on either the sensitivity of the efflux response to the drug or on the maximum efflux, but that the sensitivity of the contractile response showed an optimum at about 2·5 mM and fell sharply on either side. The maximum contractile response increased monotonically with the calcium concentration.

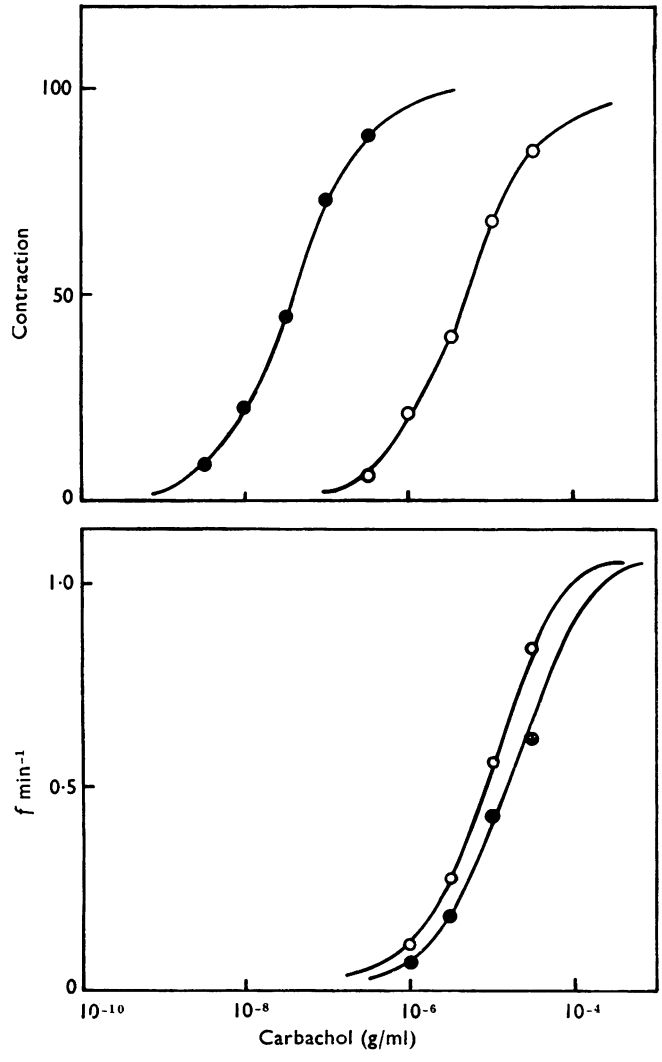

FIG. 2. Dose response curve as in Fig. 1 but calcium concentrations were 2·5 mM (●——●) and 1·25 mM (○——○).

Calcium in this range was also without effect on the resting Rb efflux (Table 1).

Influence of magnesium on the responses of the longitudinal muscle of the ileum

When the calcium concentration was maintained at 2·5 mM and the magnesium concentration doubled to 4·8 mM a decrease in sensitivity of the contractile response to carbachol was seen and was of the same order as that produced by the increase in calcium. Likewise a reduction in magnesium reduced the sensitivity to carbachol (Fig. 4). Again like calcium, magnesium was without effect on the efflux response. The only qualitative difference between the ions was that changes in magnesium had no effect on the maximum contractile response.

TABLE 1. *Effect of changed calcium concentration on the resting rubidium efflux*

Ca (mM)	Efflux (min^{-1})
1·25	0·007±0·001
2·5	0·008±0·001
5·0	0·007±0·002
10·0	0·009±0·001
15·0	0·009±0·002

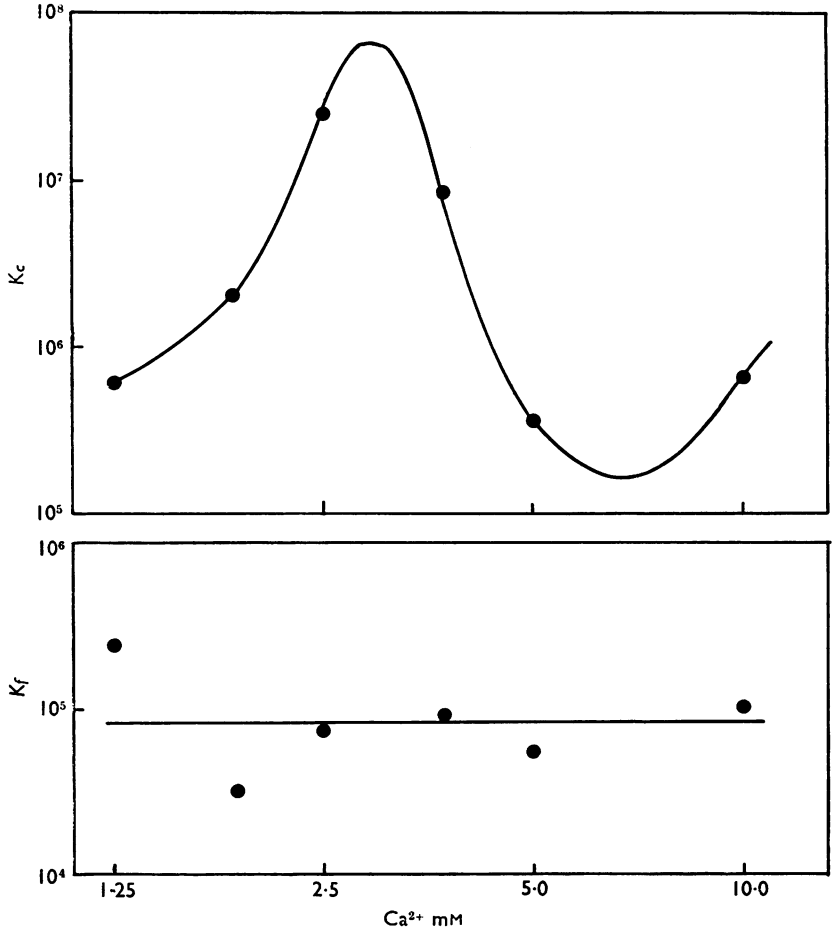

FIG. 3. Apparent affinity constants for carbachol in producing contractile response (K_c) and efflux (K_f) as a function of Ca concentration at fixed Mg concentration (2·4 mM). Abscissa: Ca concentration. Ordinates: apparent agonist affinity constants.

A comprehensive study of the effects of simultaneous variations in calcium and magnesium concentrations on the contractile response is presented in Table 2. The results are difficult to grasp in this form but are made clearer in Fig. 5 where they are presented as a two dimensional contour map. It can be seen from this map that there are optimum combinations of calcium and magnesium which differ according to whether the concentration of calcium is above or below about 2·5 mM. Above 2·5 mM the optimum is attained when the ratio Ca/Mg is constant and

TABLE 2. *Values of* K_c (M^{-1}) *as a function of calcium and magnesium concentrations*

Ca (mM) Mg(mM)	1·25	1·88	2·50	3·75	5·0	10·0
1·2	$4{\cdot}22\pm10^6$ ($\pm1{\cdot}2$)	—	$6{\cdot}15\pm10^6$ ($\pm0{\cdot}72$)	—	$5{\cdot}67\pm10^5$ ($\pm0{\cdot}21$)	—
2·4	$6{\cdot}11\pm10^5$ ($\pm1{\cdot}27$)	$2{\cdot}04\pm10^6$ ($\pm0{\cdot}18$)	$2{\cdot}56\pm10^7$ ($\pm1{\cdot}34$)	$8{\cdot}34\pm10^6$ ($\pm1{\cdot}32$)	$2{\cdot}88\pm10^5$ ($\pm0{\cdot}4$)	$6{\cdot}66\pm10^5$ ($\pm1{\cdot}24$)
4·8	$5{\cdot}28\pm10^7$ ($\pm0{\cdot}40$)	—	$5{\cdot}98\pm10^5$ ($\pm1{\cdot}45$)	—	$1{\cdot}69\pm10^7$ ($\pm0{\cdot}30$)	$1{\cdot}83\pm10^6$ ($\pm0{\cdot}12$)
9·6	$3{\cdot}44\pm10^5$ ($\pm0{\cdot}10$)	—	$7{\cdot}77\pm10^5$ ($\pm0{\cdot}28$)	—	$6{\cdot}24\pm10^5$ ($\pm2{\cdot}25$)	$4{\cdot}41\times10^5$ ($\pm0{\cdot}97$)

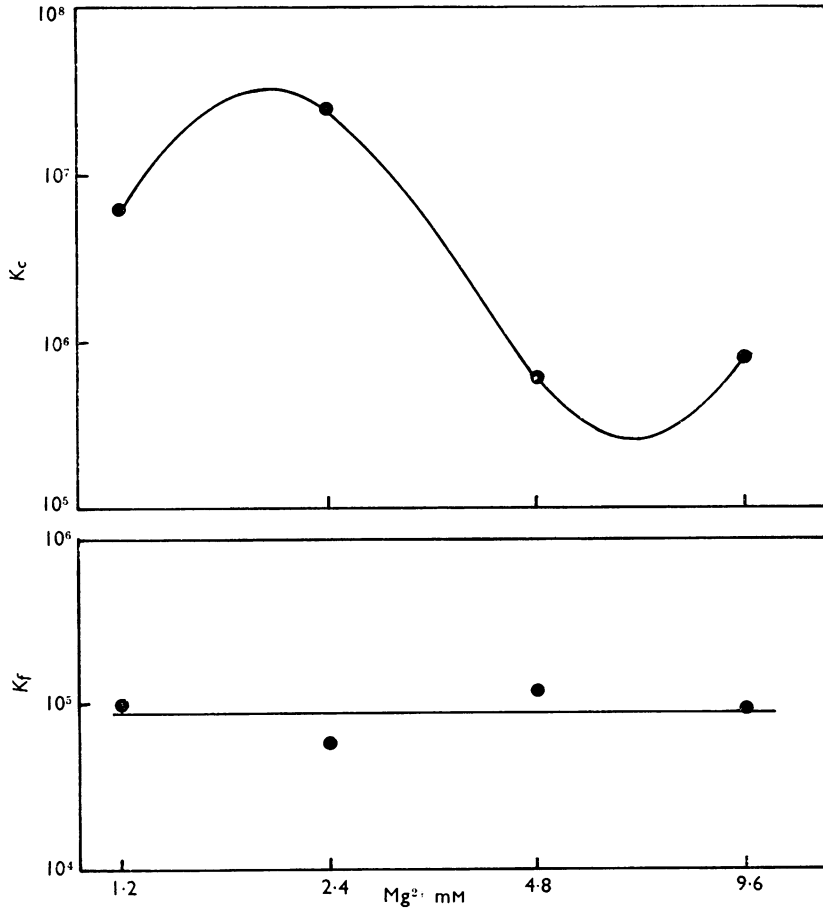

FIG. 4. Apparent affinity constants for carbachol as in Fig. 3 but at a fixed Ca concentration (2·5 mM) and variable Mg concentrations. Abscissa: Mg concentration. Ordinates: apparent agonist affinity constants.

approximately equal to 1. Below 2·5 mM calcium there is an optimum when the sum of Mg + Ca is about 5·5 mM.

Influence of calcium on responses in the taenia coli

The effect of raising the calcium concentration was much smaller than in the ileum. At 2·5 mM the value of K_c was $4{\cdot}3 \times 10^7$ M^{-1}; raising calcium to 5·0 mM reduced this to $1{\cdot}69 \times 10^7$ M^{-1} and even 15·0 mM calcium reduced K_c only to $1{\cdot}43 \times 10^7$ M^{-1}, still leaving a large K_c/K_f ratio of 208. On the other hand, reducing calcium to 1·25 mM lowered K_c to $7{\cdot}6 \times 10^5$ M^{-1} and the K_c/K_f ratio to 12. These results are summarized in Table 3. No experiments were carried out with changed magnesium concentrations. It appears that the taenia is much less sensitive to increased calcium than the ileum.

TABLE 3. *Effect of changing the calcium concentration on the response of taenia coli to carbachol*

Ca (mM)	K_c (M^{-1})	K_f (M^{-1})	K_c/K_f	f_{max} min^{-1}
1·25	$7{\cdot}57 \pm 0{\cdot}73 \times 10^5$	$6{\cdot}37 \pm 0{\cdot}27 \times 10^4$	12	1·07
2·5	$4{\cdot}30 \pm 0{\cdot}41 \times 10^7$	$6{\cdot}50 \pm 0{\cdot}34 \times 10^4$	650	1·12
5·0	$1{\cdot}69 \pm 0{\cdot}41 \times 10^7$	$6{\cdot}43 \pm 0{\cdot}54 \times 10^4$	260	1·09
10·0	$1{\cdot}42 \pm 0{\cdot}48 \times 10^7$	$6{\cdot}95 \pm 0{\cdot}65 \times 10^4$	199	1·14
15·0	$1{\cdot}43 \pm 0{\cdot}58 \times 10^7$	$6{\cdot}60 \pm 1{\cdot}00 \times 10^4$	208	1·16

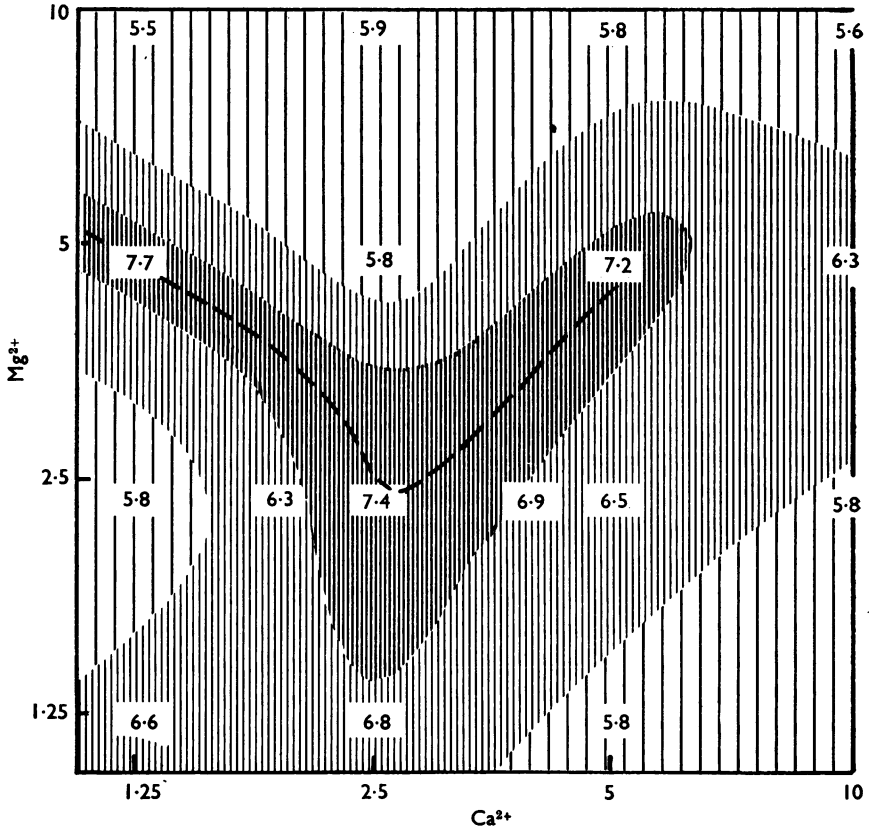

FIG. 5. Two dimensional contour map of apparent affinities for the contractile response to carbachol as a function of both Ca and Mg concentrations. (IIIII): log K_c, 7–8; (IIII): log K^c, 6–7; (III): log K_c, 5–6.

Influence of raised calcium on other muscarinic agonists

The effect of raising calcium to 5·0 mM was tested on the actions of five other muscarinic agonists. Effects comparable with those obtained with carbachol were seen with dilvasene and oxotremorine-M. A smaller change was seen with methylfurmethide but no effect at all with pentyltrimethylammonium or hexyltrimethylammonium. The latter are agonists in which there is little difference between K_c and K_f. In 5 mM calcium the K_c/K_f ratio for all six agonists was in the range 1·8–7·0 (Table 4), averaging 4·1.

Influence of raised calcium on the behaviour of antagonists

Raising or lowering the calcium concentration had no effect on the affinity constant of atropine in antagonizing the effects of carbachol on either efflux or contraction (Table 5).

TABLE 4. *Effect of raised calcium on other muscarinic agonists*

	2·5mM Ca			5·0 mM Ca		
	$K_c(M^{-1})$	$Kf(M^{-1})$	K_c/K_f	$K_c(M^{-1})$	$Kf(M^{-1})$	K_c/Kf
Carbachol	$2{\cdot}56\times10^{7}$	$7{\cdot}71\times10^{4}$	331	$2{\cdot}88\times10^{5}$	$5{\cdot}7\times10^{4}$	5·0
Dilvasene	$5{\cdot}29\times10^{5}$	$1{\cdot}05\times10^{4}$	50	$1{\cdot}09\times10^{5}$	$1{\cdot}57\times10^{4}$	7·0
Oxotremorine M	$9{\cdot}81\times10^{6}$	$7{\cdot}68\times10^{5}$	13	$6{\cdot}56\times10^{5}$	$2{\cdot}60\times10^{5}$	2·5
Methylfurmethide	$8{\cdot}01\times10^{5}$	$1{\cdot}63\times10^{5}$	4·9	$2{\cdot}34\times10^{5}$	$6{\cdot}98\times10^{4}$	3·4
Pentyl TMA	$7{\cdot}10\times10^{3}$	$2{\cdot}39\times10^{3}$	3·0	$8{\cdot}69\times10^{3}$	$4{\cdot}89\times10^{3}$	1·8
Hexyl TMA	$7{\cdot}19\times10^{3}$	$3{\cdot}73\times10^{3}$	1·9	$6{\cdot}89\times10^{3}$	$3{\cdot}14\times10^{3}$	2·2

TABLE 5. *Antagonist affinity of atropine for ileum*

Ca (mM)	For efflux $K_A(M^{-1})$	For contraction $K_A(M^{-1})$
1·25	$6{\cdot}90\pm0{\cdot}82\times10^{8}$	$6{\cdot}83\pm0{\cdot}27\times10^{8}$
2·5	$7{\cdot}53\pm1{\cdot}08\times10^{8}$	$6{\cdot}54\pm0{\cdot}98\times10^{8}$
5·0	$7{\cdot}82\pm1{\cdot}21\times10^{8}$	$6{\cdot}13\pm1{\cdot}04\times10^{8}$

TABLE 6. *Spare receptor ratios estimated with benzilylcholine mustard*

	Spare receptor ratio	
Ca (mM)	For efflux	For contraction
2·5	273±47	384±46
5·0	360±23	353±88

TABLE 7. *Effect of calcium on dibenamine blockade*

Ca (mM)	K_f (M^{-1})	K_f limit* (M^{-1})	K_c (M^{-1})	K_c limit* (M^{-1})
2·5	$7{\cdot}71\times10^{4}$	$2{\cdot}32\times10^{4}$	$2{\cdot}56\times10^{7}$	$2{\cdot}26\times10^{4}$
5·0	$5{\cdot}70\times10^{4}$	$2{\cdot}44\times10^{4}$	$2{\cdot}88\times10^{5}$	$2{\cdot}72\times10^{4}$

* Limiting value obtained when the maximum response had been reduced by dibenamine

TABLE 8. *Effect of sodium and sucrose concentration on the responses to carbachol*

Na (mM)	Sucrose (mM)	K_c (M^{-1})	K_f (M^{-1})	f_{max} (min^{-1})	K_c/Kf
144	0	$2{\cdot}56\times10^{7}$	$7{\cdot}71\times10^{4}$	0·910	331
83	61	$3{\cdot}61\times10^{5}$	$9{\cdot}90\times10^{4}$	1·12	3·7
144	88	$4{\cdot}93\times10^{5}$	$1{\cdot}41\times10^{5}$	0·69	3·5
144	117	$9{\cdot}94\times10^{5}$	$1{\cdot}2\times10^{5}$	0·69	8·2
144	176	$4{\cdot}93\times10^{5}$	$1{\cdot}56\times10^{5}$	1·04	3·2

The effectiveness of benzilylcholine mustard was also unaffected as was the estimate of the spare receptor ratio obtained with this agent (Table 6).

The effects of dibenamine were interesting. We had found previously that in 2·5 mM calcium dibenamine caused a parallel shift of the contraction dose-response curve before reducing the maximum, whereas it caused virtually no shift in the efflux curve. In the presence of raised calcium (5 mM) no change in the limiting value of either K_f or K_c was found, but because the contraction dose-response curve before exposure to dibenamine had been shifted so much to the right the parallel shift obtained with dibenamine was much reduced (Table 7).

Influence of lowered sodium and raised osmolarity

Reduction of sodium to 83 mM (replacing sodium chloride by an isosmotic amount of sucrose) had an effect similar to a change in calcium concentration, K_c being reduced to $3{\cdot}61 \times 10^5$ M^{-1} without significant change in K_f. A similar effect was produced by a modest increase in osmolarity (Table 8).

Discussion

The results reported in this paper showing that reduction of calcium concentration decreases the potency of muscarinic drugs as measured by their ability to produce a contraction response of the ileum are entirely in accord with the results of Paton & Rothschild (1965). The effects due to raised calcium concentrations and to changes in magnesium, sodium and osmolar concentrations have not been reported previously. The interplay of these factors make it clear that the control of the sensitivity of the contraction response is not just dependent on calcium concentration but that several processes must be involved. The insensitivity of the efflux response to these same factors is in striking contrast.

In a previous paper (Burgen & Spero, 1968) we concluded on the basis of structure-activity relationships of agonists, that there are probably two distinct populations of receptors concerned with the efflux and contractile responses. It is an interesting feature of the experiments reported here, that when the concentration of calcium is not optimal the discrepancy between the sensitivity of the two responses to a particular agonist is virtually abolished and hence the structure-activity relationships for the two responses are no longer distinguishable. These considerations suggest an alternative explanation of the original results, namely that both responses are due to activation of a single receptor by alteration of its conformation. The concept of efficacy which is an essential feature of the occupation theory of drug action, already implies that there is a parameter of drug-receptor interaction that regulates the efficiency of the drug-receptor complex in response production. In terms of a conformation alteration this means that differences in efficacy are to be correlated with different conformation changes. To explain differential effects on the contraction and efflux responses it is only necessary to postulate in addition that the conformational parameter determining the contraction response is not the same as that determining the efflux response—that is these responses are coupled to different domains on the receptor molecule and hence may have different efficacies.

This concept of a single receptor mediating both responses would explain why antagonist affinities and spare receptor capacity as measured with benzilylcholine mustard are identical for the two responses.

The nature of the coupling processes between the agonist-induced change in receptor conformation and the measured responses is unknown. There is clearly a fundamental difference between some of the coupling responses involved in the contractile response and in the efflux response. It is possible that the coupling process for the contractile process involves low resistance cell-cell connections, since such a functional syncytium has been shown to be necessary for the production of a synchronous contraction (Loewenstein, 1966).

The changes in cation concentrations and osmolarity that altered the contraction sensitivity are similar to those that have been shown to modify cell-cell coupling in other systems. However, it is not possible to explain the effects on contraction by this mechanism alone.

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(*Received May* 13, 1970)