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The effect of gallamine, gallopamil and nifedipine on responses to acetylcholine and carbachol in the taenia of the guinea-pig caecum

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1 The effects of gallamine, gallopamil and nifedipine on isotonic contractions of the isolated taenia of the guinea-pig caecum produced by acetylcholine (ACh) or carbachol (CCh) were investigated.

2 Gallamine (0.1 to 0.3 mM) inhibited contractions produced by CCh more than those produced by ACh. The difference was still present after pretreatment of the tissue with paraoxon (10 μM for 20 min) to inhibit cholinesterases or in experiments carried out in the presence of tetrodotoxin (0.3 μM) to exclude possible ganglionic stimulation by the agonists.

3 Gallopamil or nifedipine selectively inhibited the tonic response to ACh in the absence or presence of paraoxon. The phasic response to ACh or the tonic response to CCh (0.1 or 1 μM) was much less affected.

4 Reduction of the Ca^{2+} content of the bath medium reduced phasic and tonic responses to ACh more than the tonic response to CCh.

5 These results suggest that there are differences in the interaction of ACh and CCh with muscarinic receptors in this muscle.

Introduction

The taenia of the guinea-pig caecum has frequently been used to investigate the ionic and electrophysiological basis for the action of cholinomimetics and other agonists on ileal smooth muscle. Cholinomimetics, such as carbachol (CCh) and acetylcholine (ACh), produce contraction of this muscle by activation of muscarinic receptors (Akubue, 1966; Hobbiger *et al.*, 1969; Nasu & Urakawa, 1973; Casteels & Raeymakers, 1979) and this leads to depolarization and an increased firing of action potentials before calcium is subsequently made available for contraction (Bülbring & Burnstock, 1960; Bolton, 1979). Cholinomimetics also contract the taenia after depolarization with K^+ so that other mechanisms for increasing the availability of calcium may be employed in certain circumstances (Durbin & Jenkinson, 1961a, b).

The contraction produced by cholinomimetics in ileal smooth muscle may also be differentiated further into a phasic response and a later tonic response (Ticku & Triggle, 1976; Triggle & Triggle, 1976; Rosenberger *et al.*, 1979). It has been suggested that the phasic response involves the release of a superficially bound membrane store of calcium and the tonic response is due to an increased influx of calcium across the cell membrane (Chang & Triggle, 1973; Triggle & Triggle, 1976; Ticku & Triggle, 1976; Daniel *et al.*, 1979).

Most investigators have suggested that ACh and CCh produce similar effects in the taenia and other ileal longitudinal muscle of the guinea-pig; these are based on findings of electrophysiological investigations (Bolton, 1972; Bolton & Clark, 1981) and of studies on changes in Ca^{2+} concentration (Casteels & Raeymaekers, 1979) or on K^+ fluxes (Burgen & Spero, 1968). Differences in the actions of these agonists have been attributed to the hydrolysis of ACh by esterases since the differences diminished when the enzymes were inhibited (Bolton & Clark, 1981). In this paper, it is demonstrated in the taenia that ACh and CCh exhibit different sensitivities towards gallamine or the 'calcium entry blocking drugs' nifedipine and gallopamil (D600) and these differences are still present after cholinesterase inhibition. The neuromuscular blocking drug gallamine can

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block muscarinic receptors and exhibits a selectivity for certain types of muscarinic receptors, possibly through an allosteric mechanism or by acting on muscarinic receptor subtypes (Clark & Mitchelson, 1976; Stockton *et al.*, 1983).

Methods

Pieces of taenia ca. 1 cm long were dissected from the caecum of guinea-pigs weighing 400–600 g and were set up in a 15 ml organ bath under an initial tension of 0.5 g in Tyrode solution gassed with a 95% O_2 and 5% CO_2 mixture and maintained at 37°C. The Tyrode solution had the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.21, $NaHCO_3$ 12, glucose 5.5. Responses were recorded isotonically using an isotonic lever (Hugo Sachs) coupled via a HF Modem Type 305 (Hugo Sachs) to a pen recorder.

Agonists were added to the organ bath at 10–15 min intervals and usually remained in contact with the tissue for periods of up to 4 min before being washed out. Responses were expressed as a percentage of the response to a maximal dose of CCh (3 μM). Antagonists remained in contact with the tissue for an initial equilibration period of 30 min (gallamine and atropine) or 60–90 min (gallopamil and nifedipine) and were re-added to the organ bath after each washout of the agonists. Experiments using nifedipine were conducted in a room screened from daylight and illuminated with sodium lamps. Some experiments were conducted after pretreatment of the taenia with the irreversible anticholinesterase paraoxon (10 μM for 20 min). Following treatment, the tissue was washed free of excess anticholinesterase and used without further addition of paraoxon.

A calculation based on a k_2 value of 1.1 $(\mu mol\,min)^{-1}$ for paraoxon with erythrocyte cholinesterase (Cohen & Oosterbaan, 1963) showed that the $t_{\frac{1}{2}}$ for 90% inhibition with 10 μM paraoxon is 0.23 min. Although initially there was a marked increase in the tone of preparations treated with paraoxon, after washout of the anticholinesterase the tone gradually relaxed back to the original level during the next 1 to 2 h.

To exclude neuronal stimulation, some experiments were conducted in the presence of tetrodotoxin (0.3 μM). This concentration has previously been shown to abolish responses to nicotinic agonists in the taenia (Hobbiger *et al.*, 1969).

To compare the effects of gallopamil and nifedipine on responses to ACh and CCh, experiments were conducted in which similar-sized contractions to the two agonists were produced in the same preparation. This procedure was considered necessary as the relationship between calcium release and the magnitude of contraction is not known but is probably not linear if results with depolarized smooth muscle (Van Breemen, 1977) can be extrapolated to those in 'normal' physiological solution. Two concentrations of CCh were chosen (0.1 and 1 μM), the latter producing maximal or near-maximal contraction of the taenia. The two concentrations of ACh used were 1 and 10 μM in the absence of cholinesterase inhibition while 0.1, 1 and sometimes 10 μM were used in preparations pretreated with paraoxon.

In experiments conducted with varying Ca^{2+} concentrations, responses to the agonists were obtained in a Tyrode solution containing 1.8 mM Ca^{2+} as described above for experiments with gallopamil or nifedipine. The bath solution was changed to one containing a lower Ca^{2+} concentration and the tissue was incubated with this solution for 30 min to allow equilibration before responses to the agonists were re-determined. The preparation was washed 5 times throughout the 30 min incubation period in the modified solution. Two washes were made at a one minute interval on first introducing the solution and then after 10, 20 and 30 min had elapsed. No correction was made in the ionic composition of the bathing solution for the reduction in Ca^{2+}.

Statistical comparisons were made using Student's *t* test (unpaired).

Drugs used were: acetylcholine iodide (Fluka), carbamoylcholine hydrochloride (Merck), gallamine triethiodide (May & Baker), gallopamil (D600, Knoll), histamine dihydrochloride (Merck), nifedipine (Bayer), paraoxon (E600, Bayer) and tetrodotoxin (Boehringer Mannheim).

Nifedipine was supplied as a solution (0.01%) of the following composition: polyethylene glycol 15%, ethanol (96%) 15%, water 70%. Dilutions were made in distilled water. All other dilutions of drugs were made in 0.9% w/v NaCl solution (saline).

Results

Responses to agonists

The typical effect of ACh (0.1 to 10 μM) was a rapid contraction which reached a peak within 15 s and was followed by some fading of the response before a well maintained tonic response developed (Figure 1a). After paraoxon pretreatment, both phases of the response were increased and the phasic response was often not so clearly distinguishable (Figure 1b).

Responses to CCh (0.01 to 1 μM) rarely showed a visible phasic component, the usual response being a continuously increasing tonic response which reached a maximum within 4 min (Figure 1b). Tet-

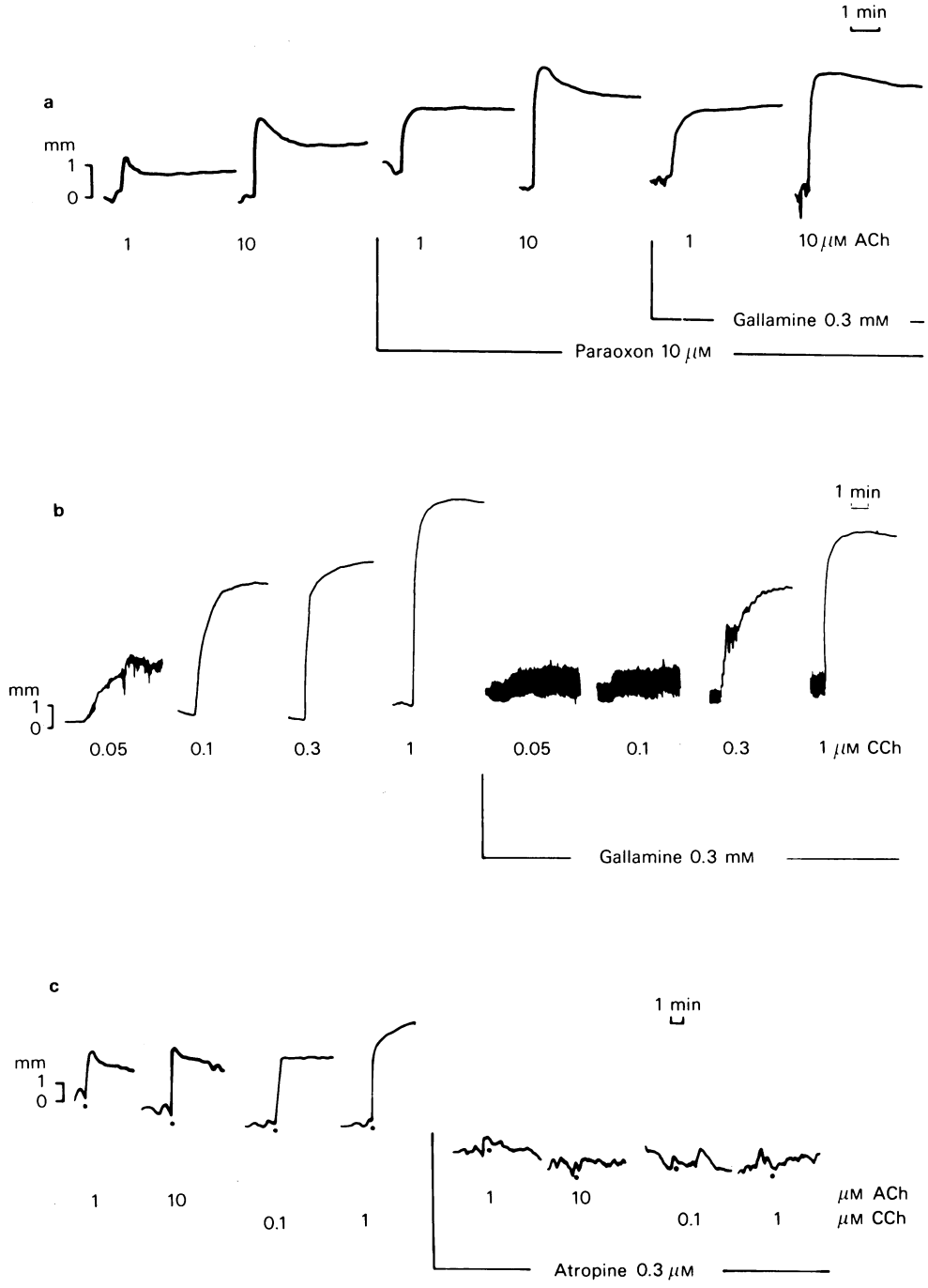

Figure 1 (a) Responses of the taenia of the guinea-pig caecum to acetylcholine (ACh) (1 and 10 μM) in the absence and presence of paraoxon (10 μM) or paraoxon plus gallamine (0.3 mM). The responses have been redrawn from original records. (b) The effect of carbachol (CCh) in the absence and presence of gallamine (0.3 mM). (c) The effect of acetylcholine (ACh) and carbachol (CCh) in the absence and presence of atropine (0.3 μM). Small variations in the resting tone may be observed from the different levels at which the responses to the agonist are initiated.

rodotoxin (0.3 μM) did not modify the responses to the two cholinomimetics.

Responses to histamine (0.1 to 10 μM) reached a maximum within 30 to 60 s and the response was maintained with little or no fading.

Effect of gallamine and atropine

Gallamine (50 to 300 μM) frequently caused an increase in the spontaneous activity of the taenia and a slight increase in tone. Both with and without paraoxon pretreatment, responses to ACh were not markedly affected by gallamine (300 μM) (Figures 1a and 2), whereas responses to CCh were inhibited (Figure 1b) and the dose-response curve shifted rightwards (Figure 3).

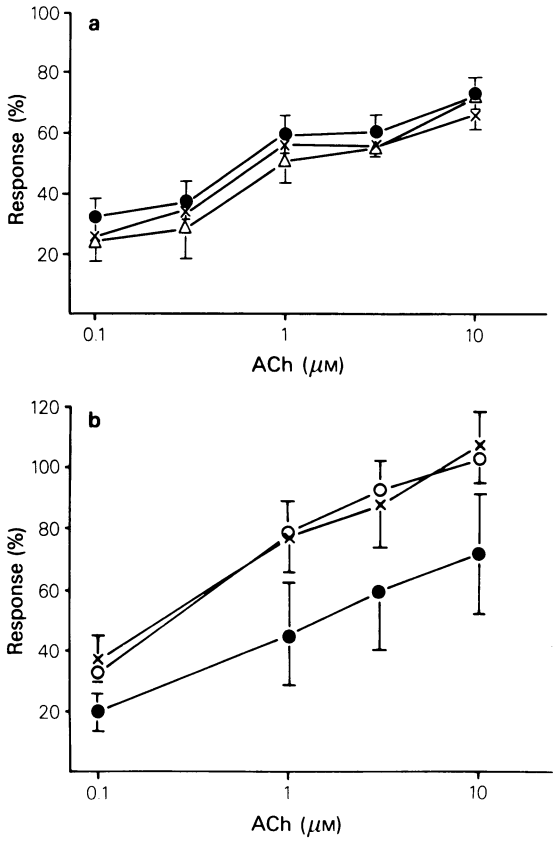

Figure 2 (a) Concentration-response relationship for the phasic response to acetylcholine (ACh) in the absence (●——●) or presence of gallamine (0.1 mM) (×——×) or (0.3 mM) (△——△) ($n=4$). (b) Concentration-response relationship for the phasic response to acetylcholine (ACh) in the absence (●——●) or presence of paraoxon (10 μM, 20 min) (×——×) or paraoxon plus gallamine (0.3 mM) (○——○) ($n=4$). Responses are expressed as a percentage of the response to carbachol (3 μM). Vertical lines show s.e.mean.

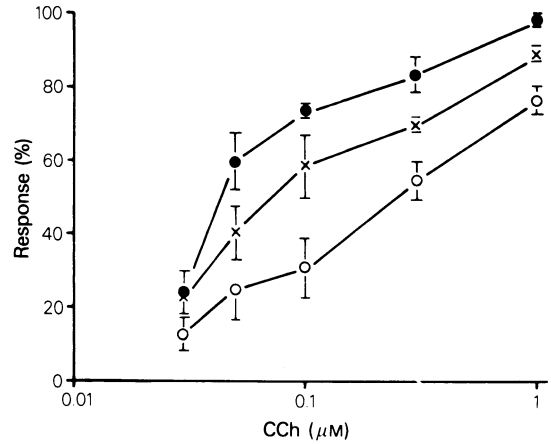

Figure 3 Concentration-response relationship for the response to carbachol (CCh) in the absence (●——●) or presence of gallamine (0.1 mM) (×——×) or (0.3 mM) (○——○). Responses are expressed as a percentage of the response to CCh (3 μM) and vertical lines show s.e.mean ($n=5$).

The presence of tetrodotoxin (0.3 μM) did not alter the effect of gallamine on responses to ACh and CCh, and gallamine (300 μM) did not affect responses to histamine.

Atropine (0.3 μM) completely abolished responses to ACh and CCh in concentrations up to 10 and 1 μM, respectively (Figure 1c).

Effect of gallopamil (D600)

Gallopamil (0.1 and 0.3 μM) reduced both the phasic and tonic responses to ACh, but the tonic response was inhibited to a greater degree as the response to ACh continued to fade during the course of the 4 min contact time with the agonist (Figure 4a). Responses to CCh (0.1 and 1 μM) were inhibited slightly by gallopamil 0.1 μM but the response to the agonist was well maintained in the presence of gallopamil. With a 3 fold increase in the concentration of gallopamil, responses to CCh also reached an initial peak and then exhibited some fading of the response during the 4 min period of contact (Figure 4a). Calculation of the magnitude of the response remaining at the end of 4 min as a percentage of the control response to the same concentration of agonist at the same point in time, showed that both low and high concentrations of the one agonist were reduced to a similar extent and that tonic responses to ACh were inhibited to a greater extent than those to CCh (Figure 5) and this difference was significant ($P<0.05$, Student's t test).

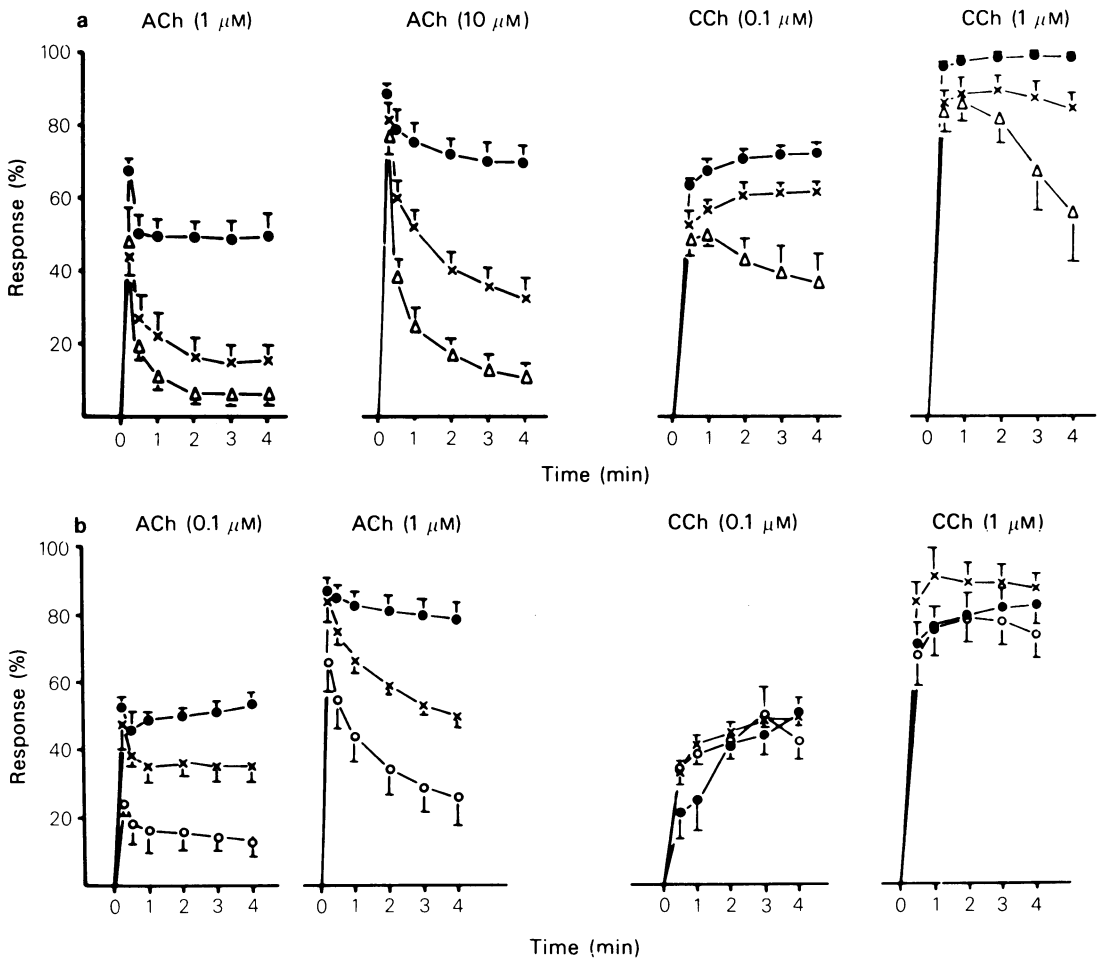

Figure 4 (a) Time course of responses to acetylcholine (ACh) 1 and 10 μM and carbachol (CCh) 0.1 and 1 μM in the absence (●——●) and presence of gallopamil 0.1 (×——×) or 0.3 μM (△——△). (b) Time course of responses to ACh (0.1 and 1 μM) and CCh (0.1 and 1 μM) in preparations pretreated with paraoxon (10 μM, 20 min) in the absence (●——●) and presence of gallopamil 30 nM (×——×) and 50 nM (○——○). Responses to both agonists obtained in the same preparations (see Methods). Responses are expressed as percentage of responses to CCh, 3 μM; (a) $n = 6$, (b) $n = 4$. Vertical lines show s.e.mean. Abscissae show time in min. (a) There is a significant difference ($P < 0.05$, Student's t test) in the magnitude of the percentage reduction in the 4 min response to CCh 0.1 μM induced by gallopamil at either concentrations compared to the corresponding reduction in the response to either ACh 1 or 10 μM.

Experiments were also conducted in paraoxon-pretreated preparations. In these preparations, similar concentrations of ACh and CCh could be used to give matched contractions. Gallopamil (0.1 and 0.3 μM) appeared more effective, in these experiments, at inhibiting the tonic responses and there was little difference between the extent of the reduction for the 2 agonists. However, lowering the concentration of gallopamil to 30 or 50 nM again led to a significantly greater depression of the tonic response to ACh than of those to CCh (Figures 4b and 5).

Effect of nifedipine

With or without paraoxon pretreatment, nifedipine 3 and 10 nM produced a greater inhibition of the tonic response to ACh than of the phasic response. Additionally, there was a greater depression of the tonic

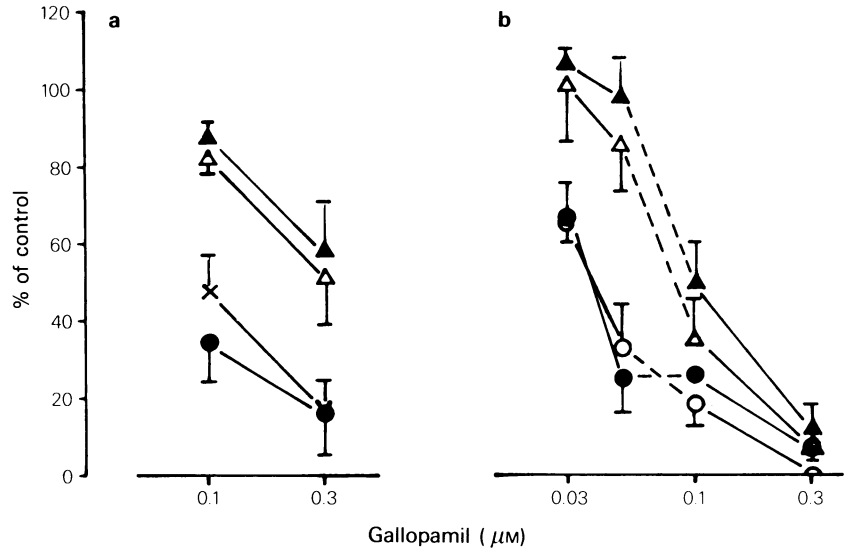

Figure 5 The effects of gallopamil on the tonic response to acetylcholine (ACh) or carbachol (CCh). The control response to any one concentration of the agonist at the end of the 4 min contact period was taken as 100%. The response remaining after 4 min contact with the agonist in the presence of gallopamil was expressed as a percentage of the control response to that concentration of agonist. Data from untreated preparations ($n = 6$) (a) or paraoxon pretreated preparations ($n = 4-5$) (b) are shown for ACh 0.1 μM (○——○), 1 μM (●——●) or 10 μM (×——×) and for CCh 0.1 μM (△——△) or 1 μM (▲——▲). Points linked with a continuous line were obtained in the same preparations. Where standard error bars are not shown they lie within the dimensions of the symbols. Ordinates: percentage of control; Abscissae: concentration of gallopamil. In preparations not pretreated with paraoxon there is a significant difference between the extent of the reduction to the two concentrations of CCh compared to those for ACh ($P < 0.05$) induced by either concentration of gallopamil (0.1 or 0.3 μM). In paraoxon-pretreated preparations, the reduction in the response to CCh 1 μM is significantly different from that to ACh 1 μM with gallopamil 0.03 ($P < 0.001$), 0.05 ($P < 0.01$) and 0.1 μM ($P < 0.05$) and the reduction in the response to CCh 0.1 μM is significantly different from that to ACh 0.1 μM with gallopamil (0.05 μM) ($P < 0.001$).

response to ACh than of that to CCh (Figure 6a). This was not as clearly evident as for gallopamil when responses at the end of the 4 min were compared (Figure 7), but if the difference for the higher concentration of each agonist is compared and then for the lower concentration in both cases the difference between the 2 agonists is significant. After paraoxon pretreatment, the differential effects of nifedipine were maintained although the concentration of ACh was now reduced ten fold.

One difference noted between the effect of nifedipine and the effect of gallopamil, particularly after paraoxon pretreatment, was that in the presence of the former, the response to CCh rapidly reached a certain level and the response was maintained more or less at that level, whereas after gallopamil the response to CCh particularly in high concentrations reached a maximum and then declined. Extending the time of contact of the agonist with the tissues to 10 min in the presence of gallopamil (plus paraoxon) did not eliminate the difference in the size of the residual tonic response for the 2 agonists, the contraction produced by ACh 1 μM being inhibited significantly more than responses to CCh 0.1 μM or 1 μM ($P < 0.01$).

Reduced Ca^{2+} content

Reducing the concentration of calcium three fold from 1.8 mM had little effect on responses to ACh (Figure 6b) but after reducing the calcium content of the Tyrode solution ten or thirty fold, both phasic and tonic responses to ACh were reduced (Figures 6b and 8). The tonic response was inhibited significantly more than the phasic response (Figure 8). Responses to CCh were also affected significantly less at the end of the 4 min period than were responses to comparable concentrations of ACh (Figures 6b and 8).

Discussion

In the taenia, gallamine was a more effective inhibitor of responses to CCh than to ACh. This was

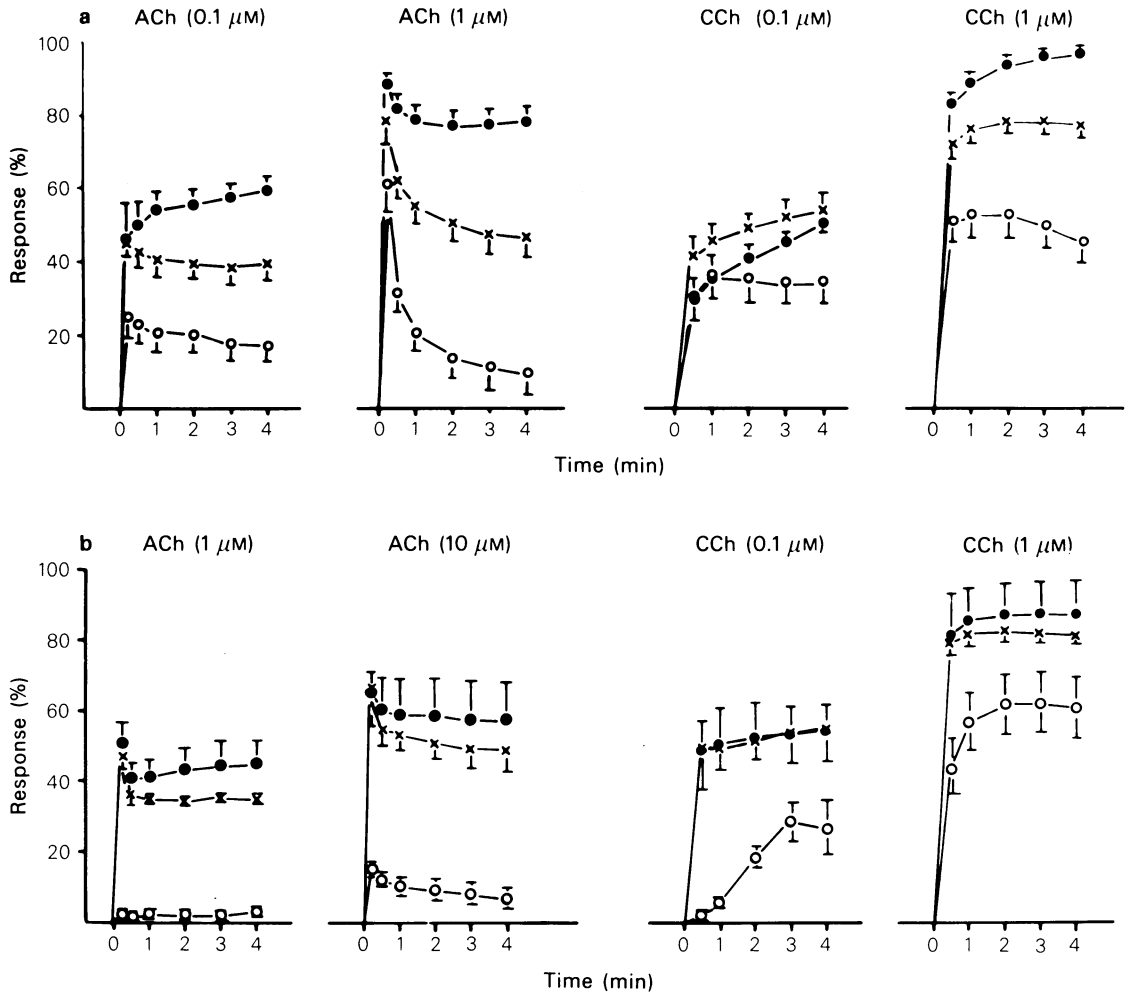

Figure 6 (a) Time courses of responses to acetylcholine (ACh) (0.1 and 1 μM) and carbachol (CCh) (0.1 and 1 μM) in preparations pretreated with paraoxon (10 μM, 20 min) in the absence (●——●) and presence of nifedipine 3 nM (×——×) and 10 nM (○——○) ($n = 5$). (b) Time courses of responses to ACh (1 or 10 μM) and CCh (0.1 or 1 μM) in Tyrode solution containing 1.8 mM Ca^{2+} (control) (●——●) 0.6 mM Ca^{2+} (×——×) or 60 μM Ca^{2+} (○——○) ($n = 4$). Other details as in Figure 4.

not due to an activation of ganglionic receptors by CCh and their subsequent blockade by gallamine as the presence of tetrodotoxin did not modify the effect of gallamine on the responses to the two agonists. Furthermore, responses to both ACh and CCh were unaffected by tetrodotoxin in accordance with previous findings (Hobbiger *et al.*, 1969; Nasu & Urakawa, 1973) and ganglion blocking activity of gallamine is only observed in other smooth muscle preparations of the guinea-pig with higher concentrations than were used in taenia (Birmingham & Hussain, 1980). Another possible reason for the difference is that the anticholinesterase activity of gallamine (Marshall *et al.*, 1980) could counteract the inhibition of responses to ACh, but pretreatment of the tissue with the irreversible anticholinesterase paraoxon did not increase the inhibitory effect of gallamine on responses to ACh.

Gallamine is known to inhibit cardiac muscarinic receptors selectively (Brown & Crout, 1970), the affinity of gallamine for cardiac muscarinic receptors being over 10 fold higher than for ileal receptors (Clark & Mitchelson, 1976), and a similar order of affinity to that obtained in guinea-pig ileum was

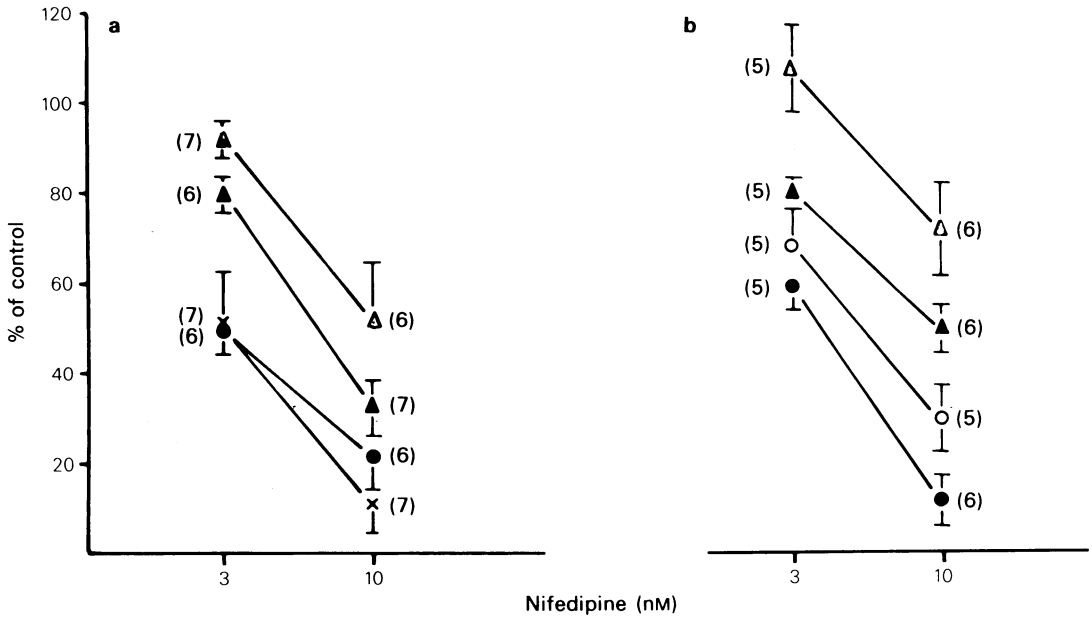

Figure 7 The effect of nifedipine on the tonic response, of untreated (a) or paraoxon-pretreated (b) preparations, to acetylcholine (ACh) or carbachol (CCh). Ordinates: percentage of control response; abscissae: concentration of nifedipine. The number of experiments associated with each point is shown in parentheses. Data are shown for ACh 0.1 μM (○——○), 1 μM (●——●) or 10 μM (×——×) and for CCh 0.1 μM (△——△) or 1 μM (▲——▲). Other details as in Figure 5. In preparations not pretreated with paraoxon, the percentage reduction in the response to CCh 0.1 μM is significantly different from that to ACh 1 μM after nifedipine 3 nM ($P<0.01$) and the reduction in the response to CCh1 μM is significantly different from that to ACh 10 μM after nifedipine 3 nM ($P<0.01$) or 10 nM ($P<0.05$). In paraoxon pretreated preparations, the percentage reduction in the response to CCh 0.1 μM is significantly different from that to ACh 0.1 μM after either concentration of nifedipine ($P<0.05$ for both) and the reduction in the response to CCh 1 μM is significantly different from that to ACh 1 μM after either concentration of nifedipine ($P<0.01$ for both).

found in the taenia with CCh as the agonist. Apart from a cardioselective antimuscarinic action, there are other differences in the action of gallamine and that of competitive antagonists such as atropine, which have been ascribed to an allosteric inhibition of the receptor by gallamine (Clark & Mitchelson, 1976; Stockton *et al.*, 1983) or to a competitive interaction with different affinities for two types of muscarinic receptor (Ellis & Hoss, 1982). An allosteric interaction of gallamine as opposed to a competitive interaction or the presence of different muscarinic receptor subtypes could explain the greater inhibitory effect of gallamine on responses to CCh in the taenia.

Inhibition of ion channels by gallamine, which occurs in frog skeletal muscle (Colquhoun & Sheridan, 1981), did not contribute to the blockade in the taenia as responses to histamine were unaffected by gallamine although this agonist activates the same ion channels as the cholinomimetics (Bolton *et al.*, 1981).

Evidence from binding studies in ileal tissue of multiple binding sites for muscarinic agonists (Ward & Young, 1977) and of different sources of calcium being available for contractions by cholinomimetics (Brading & Sneddon, 1980) led to the use of the 'calcium entry blockers'. If the two agonists were acting on the same receptor but on different binding sites, then there may be differences in the coupling of their receptor activation to contraction.

Although the 'calcium entry blockers' may have actions on intracellular processes involving calcium (Ziegler *et al.*, 1979; Church & Zsotér, 1980) these compounds have been used extensively in vascular smooth muscle research to distinguish between the actions of drugs using intracellular sources of Ca^{2+} for contraction from those using extracellular Ca^{2+} (Massingham, 1973; Godfraind & Miller, 1982).

Responses, or phases of responses to various agonists using extracellular Ca^{2+} for contraction, also show different sensitivities to calcium entry blocking drugs and this has been interpreted to mean that

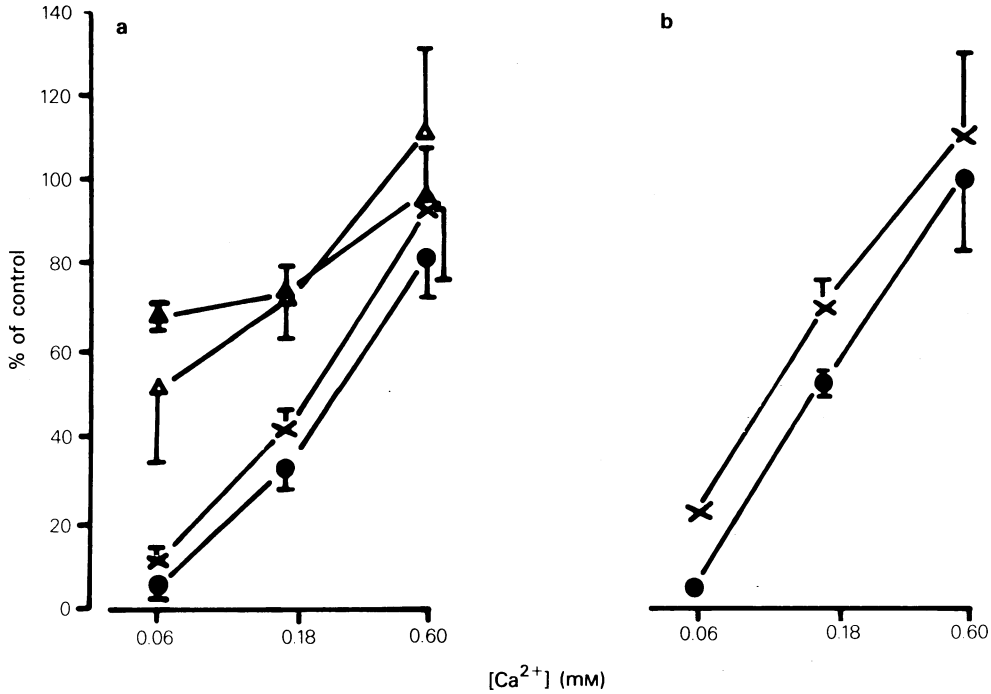

Figure 8 The effect of reducing $[Ca^{2+}]$ on tonic responses (a) and phasic responses (b) to acetylcholine (ACh) 1 μM (●——●) or 10 μM (×——×) and carbachol (CCh) 0.1 μM (△——△) or 1 μM (▲——▲) (tonic responses only). Ordinates: percentage of control response in 1.8 mM Ca^{2+}; abscissae: $[Ca^{2+}]$ of bath medium. Other details as in Figure 5 except that $n = 4$ for all points. The percentage reduction in the phasic response to ACh 1 μM is significantly different from the reduction in the tonic response when the $[Ca^{2+}]$ is lowered to one-tenth of normal ($P < 0.05$). For ACh 10 μM, the reduction is significantly different for the two phases when the $[Ca^{2+}]$ is lowered to one-tenth ($P < 0.01$) or to one-thirtieth of normal ($P < 0.05$). There is a significant difference in the percentage reduction of the 4 min response to CCh 0.1 μM compared to the corresponding response for ACh 1 or 10 μM ($P < 0.05$ for both) when the $[Ca^{2+}]$ is reduced to one-tenth of normal. When the $[Ca^{2+}]$ is one-thirtieth of normal the response to CCh 0.1 μM is only significantly different from that to ACh 1 μM ($P < 0.05$). The 4 min response to CCh 1 μM is significantly different from the corresponding response to ACh 1 μM when the $[Ca^{2+}]$ is reduced to one-tenth ($P < 0.01$) or one-thirtieth of normal ($P < 0.001$).

different Ca^{2+} channels may be utilized by different agonists (Collis & Shepherd, 1979; Godfraind & Miller, 1982; Godfraind, 1983) or by the same agonist for different phases of the response (Hurwitz *et al.*, 1980). In guinea-pig ileal longitudinal muscle, the contractions produced by cholinomimetics or K^+ are inhibited by gallopamil or nifedipine, the tonic responses being inhibited by lower concentrations, of either 'calcium entry blocker', than the phasic response (Rosenberger *et al.*, 1979). Hurwitz *et al.* (1980) have recently found that the phasic response to K^+ was inhibited by Ca^{2+} and this inhibition was antagonized by gallopamil. This may account for the difference in the sensitivity of the 2 phases of the response to the 'calcium entry blockers' although an alternative explanation is that the phasic response uses Ca^{2+} superficially bound to the cell membrane, while the tonic response depends on free extracellular Ca^{2+} (Ticku & Triggle, 1976; Rosenberger *et al.*, 1979).

In the taenia, the calcium entry blocking drugs also affected the tonic response to ACh more than the phasic response. Although gallopamil has local anaesthetic activity (Hay & Wadsworth, 1982) and can competitively displace the muscarinic receptor antagonist [^{3}H]-QNB from specific binding sites (Cavey *et al.*, 1977; Jim *et al.*, 1981), the concentrations of gallopamil employed in the paraoxon-treated taenia were over 100 fold lower than the respective EC_{50} or K_i values for these effects. Also, the concentrations were in the range shown to block both the uptake of ^{45}Ca and the tonic phase of the response induced by *cis*-dioxolane in guinea-pig ileal longitudinal muscle (Rosenberger *et al.*, 1979). Nifedipine,

which does not exhibit local anaesthetic activity (Hay & Wadsworth, 1982) and differs from gallopamil in its mode of action on smooth muscle (Jetley & Weston, 1980) and its binding site in tissues (Murphy & Snyder, 1982), also produced a selective inhibition of the tonic phase to ACh in concentrations of a similar order to those observed to inhibit Ca^{2+}-induced contractions in K^+-depolarized taenia (Spedding, 1982) and the uptake of $^{45}Ca^{2+}$ induced by *cis*-dioxolane in ileum (Rosenberger *et al.*, 1979).

The finding that equivalent tonic contractions produced by carbachol were approximately 3 fold less sensitive to gallopamil or nifedipine (see Figures 5 and 7) than those produced by ACh and required a greater decrease in the calcium concentration to produce equivalent inhibition suggests that the two agonists activate the receptor and hence the release of Ca^{2+} in a slightly different manner although both appear to be full agonists. If the agonists activate the receptor in an identical manner, coupling of the movement of calcium to muscarinic receptor activation would be expected to be independent of the agonist.

Recently, Cauvin *et al.* (1982) suggested that it is important that equi-effective responses of agonists be compared when attempting to detect differences in their sensitivity to calcium entry blocking drugs or to variations in the calcium concentration. In the taenia, two concentrations each of ACh and CCh were chosen to produce comparable contractions and furthermore, the greater effectiveness of the 'calcium entry blockers' on the tonic response to ACh compared to CCh was evidently independent of concentration when low concentrations of gallopamil were used. This was not due to the use of an arbritary 4 min cut-off point because the same results were obtained when responses to the agonists were maintained over a 10 min period.

Brading & Sneddon (1980) suggested that CCh may produce contraction of the taenia in three ways. Low concentrations of CCh ($< 1\ \mu M$) produce a contraction by increasing spike frequency and this is the main source of Ca^{2+} for these concentrations of agonist. Higher concentrations of CCh produce an initial transient peak of contraction, of ca.5 min duration, by releasing Ca^{2+} from an internal store. Following depletion of the store over the course of 5 min, the tension declines to a new level which is maintained by the continuous influx of membrane-bound Ca^{2+} by a potential-dependent mechanism which may be blocked by gallopamil ($0.2\ \mu M$). Under this scheme the responses to the two concentrations of CCh employed herein may have involved different mechanisms as part of the response to CCh $1\ \mu M$ could have been due to release of Ca^{2+} from the internal store. However, responses to the higher concentration of CCh could be maintained for 10 min without significant 'fading' and each of the calcium entry blockers had essentially similar effects on the maintained responses to the high and low concentrations of CCh, suggesting that both concentrations were acting by similar mechanisms.

Thus, in conclusion, the results with gallamine and the calcium entry blockers exposed differences in the interaction of ACh and CCh with muscarinic receptors in the taenia. At the present time it is speculative whether there are different types of muscarinic receptors in this tissue or whether the differences can be explained in terms of differences in the binding of these agonists with the one receptor.

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