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THE INHIBITORY EFFECT OF GALLAMINE ON MUSCARINIC RECEPTORS

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1 The inhibitory effect of gallamine (1.1 μM–1.1 mM) on negative inotropic responses to acetylcholine (ACh) or carbachol (CCh) was investigated in isolated electrically stimulated atria of the guinea-pig. Gallamine caused parallel rightward shifts of the dose-response curves to the agonists, with no depression of the maximal response.

2 Gallamine (0.11–1.1 mM) produced a greater degree of antagonism towards CCh than towards ACh. With either agonist, the degree of antagonism produced by gallamine in high concentrations was less than that expected for a competitive antagonist.

3 Similar findings were made when either negative inotropic or chronotropic responses were recorded in spontaneously beating guinea-pig atria. The inhibitory effect of gallamine against the negative inotropic response to cholinomimetics in electrically stimulated atria was not altered either in the presence of propranolol (17 μM) or in atria obtained from guinea-pigs pretreated with di*iso*propylphosphorofluoridate (DFP) (12.5 μmol/kg, in divided doses over 3 days).

4 When ACh was used as the agonist, combination of gallamine with atropine (0.05–0.4 μM) produced dose-ratios which were less than expected for combination of two competitive antagonists. The same phenomenon was observed in atria obtained from guinea-pigs pretreated with DFP.

5 It is suggested that the antagonism produced by gallamine is a type of non-competitive inhibition, which has been termed 'metaffinoid antagonism'. An antagonist of this type allosterically alters the affinity of the agonist for its binding site, rather than changing the effectiveness of the agonist-receptor interaction.

Introduction

It has been reported that when gallamine is used clinically as a neuromuscular blocking agent, it produces tachycardia if used in conjunction with certain types of anaesthetics (Doughty & Wylie, 1951). Investigation of this effect has yielded various possible explanations. Brown & Crout (1966; 1968; 1970) showed that gallamine had a positive inotropic effect on mammalian cardiac muscle *in vitro* which was abolished by propranolol, or by pretreatment with reserpine. They suggested that gallamine possessed a sympathomimetic effect which was mediated by the release of noradrenaline from cardiac adrenergic nerve endings.

Gallamine also antagonizes negative inotropic and chronotropic effects produced by preganglionic vagal stimulation of the heart (Della Bella, Rognoni & Gopal, 1961; Brown & Crout, 1968). Della Bella *et al.* (1961) explained this effect as being due to a ganglion blocking action of gallamine in the heart. However, Riker & Wescoe (1951) showed that gallamine also inhibited acetylcholine (ACh) or methacholine-induced bradycardia. They postulated that the action of gallamine on the heart was like that of atropine, but it was a much less potent antagonist at other muscarinic sites.

More recent studies by Rathbun & Hamilton (1970) further emphasized the specificity of gallamine for the cholinoceptors of the heart. They supported the suggestion of an atropine-like action for gallamine, as the effects produced by gallamine fulfilled their requirements for competitive antagonism. Since atropine does not possess the selectivity for the heart exhibited by gallamine, it was decided to re-investigate the anti-muscarinic effects of gallamine and to elucidate further the nature of its antagonism of cholinomimetics.

Methods

Guinea-pig isolated atria

Spontaneously beating atria, or electrically stimulated left atria were mounted in a 10 ml organ bath filled with McEwen's solution (McEwen, 1956) maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Left

atria were stimulated by a square wave stimulator at supramaximal voltage, 2 ms pulse duration and a frequency of 3 Hz. Inotropic responses of the atria were recorded under a resting tension of 1 g via a Grass force-displacement transducer, FT 03C, on a Grass model 79 polygraph. In some experiments with spontaneously beating atria, chronotropic responses were monitored by a model 7P4D tachograph attachment on a polygraph.

Other tissues

Guinea-pig isolated bladder and ileum were mounted in a 10 ml organ bath, under the same conditions as the atria. Contractions of the tissue were recorded by a force-displacement transducer on a polygraph.

Di-isopropylphosphorofluoridate (DFP) treatment

Guinea-pigs were pretreated with subcutaneous injections of DFP for 3 consecutive days before use of the atria. Solutions of DFP in 0.9% w/v NaCl solution (saline) were prepared immediately before injection, each animal receiving 5 μmol/kg on the first and third day and 2.5 μmol/kg on the second day. On the fourth day, 24 h after the last injection, animals were killed and the atria removed for isolated tissue experiments or cholinesterase determinations.

Cholinesterase determinations

Cholinesterase activity was determined by the pH-Stat method, using a Radiometer Automatic Titrator as described previously (Clark & Mitchelson, 1974). The substrate used was ACh (1 mM) and the titrant was NaOH (5 mM). In experiments to determine the anticholinesterase activity of gallamine, gallamine was incubated with the tissue for 20 min before the addition of substrate. Results are expressed as μmol NaOH g^{-1} min^{-1}.

Experimental design

In all experiments agonists were added for a period sufficient to ensure that the response to the administered concentration of drug had reached its maximum. For acetylcholine and adenosine triphosphate (ATP) a contact time of up to 1 min with the tissue was usually found sufficient together with a cycle time of 3 minutes. For CCh a contact time of 2 min was usually required with a cycle time of 5 minutes.

With these regimens, constant responses to agonists were obtained at least in duplicate. Responses were considered constant when 2 responses expressed as % inhibition differed by no more than 5%. Constant responses to at least 3 concentrations of agonist lying on the linear portion of the log dose-response line were obtained before addition of any antagonist. Following incubation with antagonists, responses to agonists were again obtained in duplicate, the duplicate response being recorded 15–30 min after the initial response. This procedure ensured that equilibrium conditions had been reached in the presence of the antagonist. The initial incubation period with the antagonist was 60 min for atropine and 20 min for gallamine or homatropine. The antagonist was replaced in the tissue bath fluid immediately after washing of the tissue. In experiments carried out in the presence of propranolol, the adrenoceptor antagonist was added to the bathing fluid reservoir at the beginning of the experiment.

In experiments involving combination of gallamine with atropine, responses in the presence of atropine alone were always determined first after establishment of a dose-response line to the agonist. Particular care was taken to ensure that the dose-ratio produced by atropine was determined under equilibrium conditions as described above. Gallamine (0.11 mM) was then added and following a further 20 min incubation, a third dose-response line was determined under equilibrium conditions in order to calculate the dose-ratio produced by the combination of the two antagonists.

In isolated bladder and ileum experiments, at least 3 concentrations of the agonist which produced between 20% and 80% of the maximum contractile response of the tissue were employed, both in the control period and after the addition of the antagonist.

Dose-ratio determinations

Linear regression lines were fitted by the method of least squares to the linear portion of the log dose-response relationship both in the control period and when the antagonist was present. Dose-ratios were obtained by calculating the concentration of agonist producing 50% inhibition of the contraction or rate of the atria (or 50% of the maximum contractile response in bladder and ileum) in the absence and presence of antagonist.

Statistical comparisons

Student's *t* test (2-tailed) was used to compare groups of data, unless otherwise stated.

Drugs

The drugs used were: acetylcholine chloride (Sigma), adenosine-5′-triphosphate (ATP) (Boehringer), atropine sulphate (Knoll, Sigma), carbachol (CCh) (Koch-Light), di*iso*propylphosphorofluoridate (DFP) (Sigma), gallamine triethiodide (May & Baker), homatropine hydrobromide (Sigma), mecamylamine hydrochloride (Merck, Sharp & Dohme), nicotine hydrogen tartrate (BDH), propranolol hydrochloride (ICI).

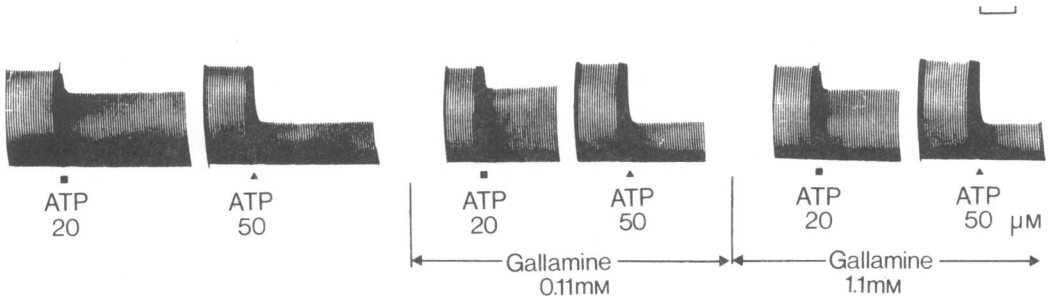

Figure 1 The effect of gallamine 0.11 and 1.1 mM on the negative inotropic response to adenosine-5'-triphosphate (ATP) 20 μM (■) and 50 μM (▲) in an electrically stimulated left atrium of the guinea-pig. Time marker indicates 4 s at fast recording speed and 24 s at slow recording speed.

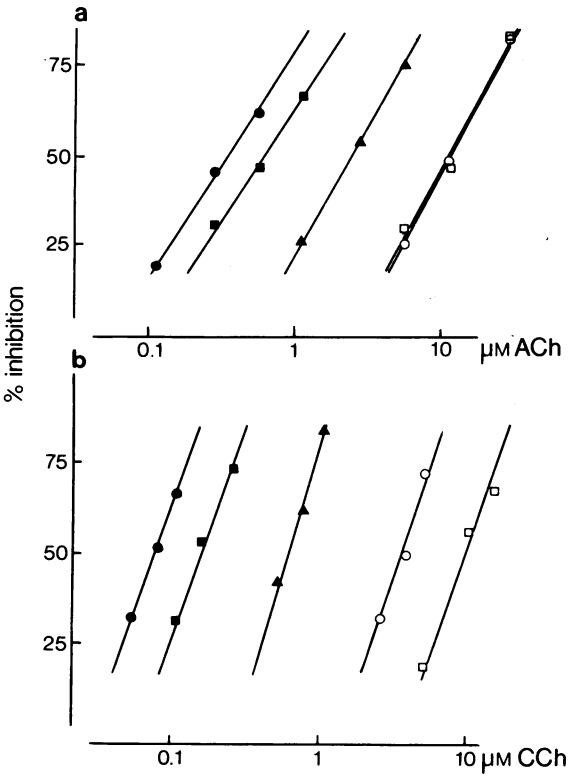

Figure 2 The typical effect of gallamine on negative inotropic responses to (a) acetylcholine (ACh) and (b) carbachol (CCh) in electrically stimulated isolated atria of guinea-pig. Graphs are the results from a single experiment with each agonist and show the parallel displacement of the dose-response line to the agonist by increasing concentrations of gallamine (1.1 μM–1.1 mM). The points show the dose-response relationship obtained for each agonist in the absence of gallamine (●), and in the presence of gallamine 1.1 μM (■), 11 μM (▲), 0.11 mM (○) and 1.1 mM (□). The slopes of the regression lines obtained with each agonist in the presence of gallamine do not differ significantly ($P > 0.05$) from those obtained for each agonist in the absence of antagonist.

Results

Isolated atrial preparations

Effect of gallamine. Gallamine produced a transient increase in contractility which did not persist throughout the initial incubation period. With a concentration of 0.11 mM the initial increase in contractility was of the order of 10–20% but within 10–15 min the response had returned to pre-gallamine levels. The lack of any permanent effect on contractility may be seen in Figure 1. Similarly, in spontaneously beating preparations gallamine 0.11 mM produced a transient positive chronotropic response of the order of 10%.

Effect of gallamine on responses to acetylcholine or carbachol. In isolated driven left atria, gallamine (1.1 μM–1.1 mM) inhibited the negative inotropic responses to ACh or CCh. Gallamine produced parallel rightward shifts of the dose-response curve to either agonist, with no depression of the maximum response (Figure 2). However, gallamine at higher concentrations produced a lesser degree of antagonism than would be expected for a competitive antagonist, the degree of antagonism appearing to reach a limiting value at high concentrations which is more marked for ACh than for CCh. This can be readily seen when the results are plotted as a function of log [gallamine] against log (dose-ratio − 1) (Figure 3). When a linear regression line was fitted to the results for CCh, the slope of the line (0.78) was found to be significantly less ($P < 0.02$) than a theoretical value of 1.0 expected for competitive antagonism. In the case of ACh, where the slope was 0.46, the difference was highly significant ($P < 0.005$).

Similar results were obtained in spontaneously beating preparations when either inotropic or chronotropic responses were monitored. Using CCh as agonist there was no significant difference ($P > 0.05$) between the geometric mean dose-ratios obtained for negative inotropic responses in stimulated and spontaneously beating atria at any concentration of gallamine (1.1 μM–1.1 mM) investigated (Table 1).

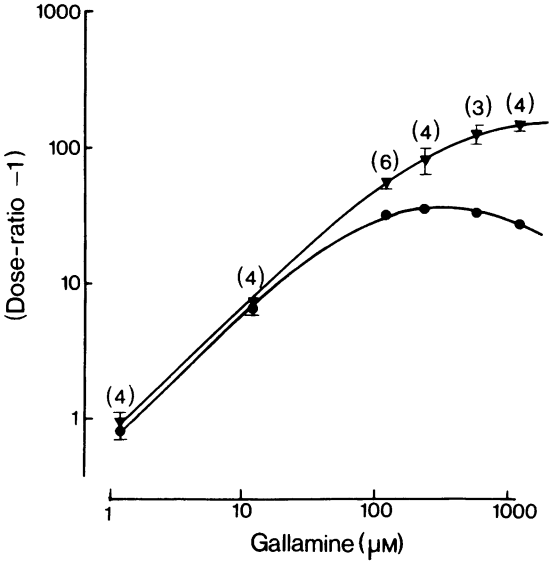

Figure 3 Effect of gallamine on negative inotropic responses of electrically stimulated atria to acetylcholine (ACh) (●) or carbachol (CCh) (▼). Each point represents the mean of the log (dose-ratio − 1) produced by a given concentration of gallamine, in four experiments with ACh as agonist. The number of experiments with CCh as agonist is shown in parentheses alongside each point. The vertical lines indicate s.e. mean, and where they are not shown they lie within the dimensions of the symbols.

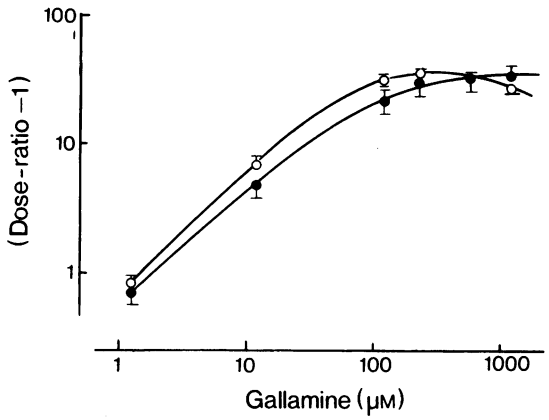

Figure 4 Comparison of the inhibitory effect of gallamine on negative inotropic responses to acetylcholine (ACh) in electrically stimulated atria from control guinea-pigs (○) and in atria from animals pretreated with di-*iso*propylphosphorofluoridate (DFP, 12.5 μmol/kg over 3 days) (●). Each point for the latter curve is the mean of 5 experiments. The curve obtained with untreated animals is that shown in Figure 3. Other details as in Figure 3.

Similarly, dose-ratios obtained with gallamine against negative chronotropic responses produced by CCh did not differ significantly ($P > 0.05$) from those obtained for the negative inotropic response in electrically stimulated atria (Table 1). With ACh as agonist, gallamine also produced similar effects on negative inotropic responses to the agonist in both preparations (Table 1).

Effect of gallamine on responses to nicotine. Gallamine (1–11 μM) did not affect the positive inotropic response of electrically stimulated atria to nicotine (6–30 μM).

Effect of gallamine on responses to ATP. Gallamine in concentrations up to 1.1 mM had no effect on negative inotropic responses to ATP (0.01–1 mM) (Figure 1).

Table 1 Comparison of the dose-ratios obtained with gallamine using either acetylcholine (ACh) or carbachol (CCh) in atrial preparations

Preparation	*Response*	*Dose-ratio produced by gallamine 0.11 mM†*		P††
		CCh	*ACh*	
Electrically stimulated	Inotropic	60.8 (6) (47.4–77.9)	34.6 (4) (26.9–44.4)	<0.05
Spontaneously beating	Inotropic	62.2 (6) (51.5–74.9)	40.1 (6) (30.0–53.6)	<0.01
	Chronotropic	75.6 (6) (37.3–153.4)	—	

† Results are shown as geometric means (number of observations) together with the 95% confidence limits in parentheses below each mean. There is no significant difference ($P > 0.05$) between any dose-ratio obtained with any one agonist.

†† P is the probability of the difference between values for CCh and ACh.

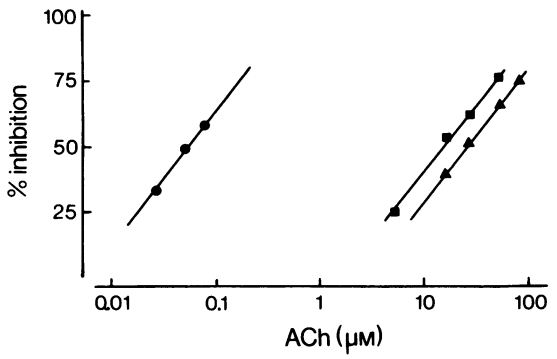

Figure 5 Illustration of the results obtained from an experiment measuring the negative inotropic response to acetylcholine (ACh) in electrically stimulated atria obtained from guinea-pigs pretreated with di-*iso*propylphosphofluoridate (DFP). Each point is the mean response from at least 2 determinations in the absence of any antagonist (●), in the presence of atropine 0.4 μM (▲) and in the presence of atropine (0.4 μM) plus gallamine 0.11 mM (■). Note that the combination of atropine plus gallamine has resulted in a lesser degree of antagonism than that occurring in the presence of atropine alone.

Effect of DFP. In electrically stimulated atria obtained from animals pretreated with DFP, responses to ACh were potentiated 3.75 fold. The mean ED_{50} (geometric) for ACh in untreated atria was 0.178 μM (95% confidence limits; 0.139–0.228, 19 experiments) and the corresponding value obtained in atria from DFP-treated animals was 0.047 μM (0.037–0.060, 28). However the dose-ratios produced by gallamine in these atria where acetylcholine was used as the agonist did not differ significantly ($P > 0.05$) at any concentration investigated from those obtained in atria from control animals (Figure 4).

Effect of propranolol. Similarly, the effect of gallamine on negative inotropic responses to CCh obtained in the presence of propranolol (17 μM) did not differ from controls.

Effect of other antagonists. The effect of homatropine (1 μM–0.3 mM) on negative inotropic responses to CCh in electrically stimulated atria was investigated. The relationship between log[homatropine] and log (dose-ratio − 1) was found to be a straight line of the type expected for a competitive antagonist, that is, with a slope (1.02) which did not differ significantly from 1.0 ($P > 0.5$). The geometric mean dose-ratios

Table 2 Dose-ratios produced by atropine (0.01–0.4 μM) and gallamine (0.11 mM) alone and in combination, with acetylcholine as agonist*

Preparation† *(n)*	*Atropine concentration (μM)*	*Atropine dose-ratio (DR_{AC})*	*Gallamine (0.11 mM) dose-ratio (DR_{AB})*	*Experimental combination dose-ratio (DR_{ACB})*	*Expected combination dose-ratio ($DR_{AC} + DR_{AB} - 1$)*	P††
Spontaneous (4)	0.01	11.5 (10.7–12.4)	40.1 (6) (30.0–53.6)	51.7 (43.2–61.8)	50.6	NS
Spontaneous (3)	0.1	100.9 (54.9–185.5)		93.9 (65.5–134.6)	140.0	<0.025
Spontaneous (6)	0.2	208.4 (186.7–232.6)		163.4 (116.3–229.5)	247.5	<0.025
Stimulated (4)	0.4	636.2 (491.8–823.0)	34.6 (4) (26.9–44.4)	363.2 (206.2–638.3)	669.8	<0.025
Stim./DFP (3)	0.05	28.4 (24.3–33.3)	24.7 (5) (12.9–47.0)	36.4 (34.8–38.0)	52.1	<0.0005
Stim./DFP (3)	0.2	247.5 (217.6–281.6)		193.7 (131.6–285.2)	271.2	<0.05
Stim./DFP (3)	0.4	547.2 (173.2–1728.0)		351.2 (172.7–714.0)	570.9	<0.05

* Dose-ratio quoted are the geometric mean dose-ratios obtained together with the 95% confidence limits in parentheses below each mean value. The dose-ratio listed for gallamine alone is the geometric mean dose-ratio (plus no. of determinations and 95% confidence limits) obtained in separate experiments. The 'expected combination dose-ratio' is the expected dose-ratio which would be obtained if both antagonists acted competitively (Paton & Rang, 1965).

† Spontaneous = inotropic response in spontaneously beating whole atria, stimulated = electrically stimulated left atria, Stim./DFP = electrically stimulated left atria obtained from animals pretreated with DFP, (*n*) = number of experiments.

†† *P* denotes the level of significance associated with a *t* test (one-tailed) for whether $DR_{ACB} < (DR_{AC} + DR_{AB} - 1)$. NS = not significant.

obtained with homatropine 1, 10, 100 and 300 μM were 15.6 (95% confidence limits 11.6–21.0, 3 experiments); 121.2 (97.3–251.9, 3); 1357 (985.8–1867, 3) and 4910 (2117–11390, 3) respectively.

Experiments with combinations of antagonists. The effect of gallamine (0.11 mM) was investigated in the presence of various concentrations of atropine in either spontaneously beating or electrically driven atria.

With ACh as the agonist and using electrically stimulated atria from either normal or DFP-pretreated animals a combination of atropine plus gallamine always produced a lesser degree of antagonism than that expected for a combination of 2 competitive antagonists (Figure 5 and Table 2). This effect was also observed in spontaneously beating atria (Table 2). In all cases this effect was most marked when higher concentrations of atropine were employed. With a concentration of 0.4 μM atropine addition of gallamine always produced a leftward shift of the dose-response line obtained in the presence of atropine (Figure 5). The same phenomenon was frequently observed with 0.1–0.2 μM atropine.

With CCh as the agonist, addition of gallamine in the presence of atropine produced a further rightward shift of the dose-response line, the resulting shift being of a similar order or less than that expected for combination of 2 competitive antagonists (Table 3).

Cholinesterase determinations

Gallamine. Gallamine 1 μM only slightly reduced the rate of hydrolysis of 1 mM ACh from 2.21 ± 0.24 (6) μmol NaOH g^{-1} min^{-1} (mean ± s.e. mean, number of observations) to 1.76 ± 0.15 (6) but gallamine 1 mM reduced the rate by 50% to 1.11 ± 0.13 (6).

DFP. The rate of hydrolysis of 1 mM ACh by atrial homogenates from untreated animals was found to be 2.15 ± 0.19 (4) μmol NaOH g^{-1} min^{-1} (mean ± s.e. mean, no. of observations). In atria obtained from DFP-treated animals the corresponding rate was 0.55 ± 0.08 (6) μmol NaOH g^{-1} min^{-1}. Pretreatment of animals with DFP resulted therefore in a 74% reduction in the rate of hydrolysis of ACh.

Other tissues

In guinea-pig isolated bladder and ileum, gallamine (1.1 μM–0.11 mM) had little inhibitory effect on contractile responses to ACh or CCh. With CCh as the agonist in ileum, gallamine (0.11 mM) produced a mean dose-ratio of 6.9 (95% confidence limits 2.5–19.3, 3 observations) and with ACh as the agonist the dose-ratio was 1.4 (0.6–3.2, 3). Similar findings were made in guinea-pig bladder. The value for CCh was 2.4 (2.1–2.8, 3) and for ACh was 1.4 (0.3–6.4, 3).

Discussion

It has been repeatedly shown that gallamine antagonizes the effects produced by ACh, methacholine (MCh) or stimulation of the vagus nerve to the heart *in vitro* (Riker & Wescoe, 1951; Della Bella *et al.*, 1961; Brown & Crout, 1968; Rathbun & Hamilton, 1970). Our investigations have reconfirmed that in isolated atria of the guinea-pig, gallamine inhibits the negative inotropic and chronotropic responses produced by ACh or CCh. Della Bella *et al.* (1961) suggested that gallamine may produce its effects by a blockade of ganglionic transmission. However, this is unlikely to be the explanation of the

Table 3 Dose-ratios produced by atropine (0.05–0.4 μM) and gallamine (0.11 mM) alone and in combination, with carbachol as the agonist in electrically stimulated atria†.

Number of experiments	*Atropine concentration (μM)*	*Atropine dose-ratio (DR_{AC})*	*Gallamine (0.11 mM) dose-ratio (DR_{AB})*	*Experimental combination dose-ratio (DR_{ACB})*	*Expected combination dose-ratio ($DR_{AC}+DR_{AB}-1$)*	P
4	0.05	74.3 (54.2–101.7)	60.8 (6) (47.4–77.9)	144.7 (83.8–250.2)	134.1	NS
4	0.1	100.2 (97.5–103.0)		113.2 (93.3–137.6)	160.0	< 0.01
3	0.2	171.6 (114.1–258.2)		195.4 (125.4–304.3)	231.4	NS
3	0.4	497.0 (411.4–600.5)		548.7 (216.0–139.4)	556.8	NS

† Same details as for Table 2.

findings reported here as gallamine in concentrations up to 11 μM, did not antagonize responses to nicotine and in preliminary experiments mecamylamine (20 μM) had no effect on responses to CCh although it completely blocked positive and negative inotropic actions of nicotine. A non-specific antagonism of cardio-inhibitory agonists is excluded also because negative inotropic responses to ATP were not affected by gallamine in concentrations up to 1.1 mM.

Gallamine thus appears to have an antimuscarinic action in the atria, and exhibits some characteristics of a competitive antagonist as it produces parallel rightward shifts of the log dose-response curve to ACh or CCh, with no depression of the maximum response. However, when the results are plotted as a function of log[gallamine] against log(dose-ratio−1) the resultant plot differs from that expected with a competitive antagonist, that is a straight line with a slope of 1.0 (Arunlakshana & Schild, 1969). In the case of gallamine, the dose-ratios produced with the higher concentrations tend towards a limiting value and the upper part of the curve no longer follows a linear relationship. This effect was observed with either electrically stimulated or spontaneously beating atria and in the latter when either inotropic or chronotropic responses were monitored. Thus it is not due to any artifact brought about by electrical stimulation or to possible interference in the magnitude of inotropic responses by concomitant changes in spontaneous rate (Koch-Weser & Blinks, 1963).

Gallamine was found to be a much less potent antagonist than atropine, a concentration of 1 mM gallamine producing similar dose-ratios to those obtained with 0.1–0.2 μM of atropine when CCh was the agonist. It may be suggested that with the higher concentrations of gallamine employed (>0.1 mM) some other action of gallamine is reducing its effectiveness as a muscarinic antagonist and thus causes the decrease in the slope of the 'Arunlakshana-Schild' curves. For example, gallamine at high concentrations might depress atrial activity non-specifically and thereby facilitate drugs such as cholinomimetics which inhibit atrial contractility. However, gallamine did not directly depress contractility or rate and furthermore had no effect on the inhibitory response to ATP. Again, it may be suggested that high concentrations of all competitive antagonists may decrease the slope of the 'Arunlakshana-Schild' plot. However, homatropine, when used in comparable concentrations (1 μM–0.3 mM) to those employed with gallamine gave rise to larger dose-ratios as well as a linear 'Arunlakshana-Schild' plot.

Gallamine has been reported to possess anti-cholinesterase activity in high concentrations (Todrick, 1954), and in the atria, gallamine 1 mM reduced the rate of hydrolysis of ACh by 50%. As such anticholinesterase activity would counteract the antimuscarinic action of gallamine at high concentrations it is of interest that responses to CCh are not potentiated by pretreatment of the animals with DFP (Madden & Mitchelson, 1975) indicating that CCh does not mediate its action by releasing endogenous ACh. Also the anticholinesterase activity of gallamine cannot account for the smaller dose-ratios obtained when ACh was the agonist in place of CCh. Pretreatment with DFP, although inhibiting hydrolysis of ACh by 74%, did not markedly affect the maximum dose-ratio produced by gallamine against ACh. However, the anticholinesterase activity of gallamine may have been responsible for the observation that concentrations of 0.5–1 mM gallamine always produced smaller dose-ratios than a concentration of 0.2 mM as this effect was not observed after DFP pretreatment (Figure 4).

Another consideration is that CCh in high concentrations may release catecholamines (Barnett & Benforado, 1966; Löffelholz, 1970). However, such a release would reduce the effectiveness of CCh in producing negative inotropic responses at high concentrations and be manifested as an apparent increase in the dose-ratios produced by gallamine. Furthermore, gallamine itself may release catecholamines (Brown & Crout, 1968), which would also increase its effectiveness as an inhibitor of cholinomimetics. The presence of propranolol did not alter the dose-ratios indicating that sympathomimetic effects were not contributing to the inhibitory activity of gallamine on responses to cholinomimetics.

One explanation of findings reported here is that gallamine is acting allosterically to inhibit ACh or CCh. Antagonists which interact at a site which is distinct from but interdependent with the binding sites for agonists and cause a reduction in the affinity of agonists for their binding sites have been discussed by Ariëns, Van Rossum & Simonis (1956; 1964). Such an effect has recently been termed 'metaffinoid antagonism' (Offermeier & van den Brink, 1974). Like competitive antagonists and unlike noncompetitive antagonists of the 'mectactoid' type (Offermeier & van den Brink, 1974) a metaffinoid antagonist produces parallel shifts of the dose-response curve to the agonist without depression of the maximal response. However, metaffinoid antagonists differ from competitive antagonists in that their inhibitory activity at high concentrations reaches a limiting value when the antagonist has occupied all its available binding sites. The fact that gallamine at high concentrations produces greater inhibition of responses to CCh than to ACh would indicate that binding of these 2 agonists to the muscarinic receptor can be differentiated by metaffinoid antagonists.

The results from experiments involving combination of atropine and gallamine are also explainable in terms of gallamine being a metaffinoid antagonist. Such an antagonist will also affect the affinity of competitive

antagonists for the muscarinic receptor and if the binding sites of competitive antagonists differ partly from those of agonists as has been suggested (Burgen, 1965) then metaffinoid antagonists may alter the affinities of agonists and competitive antagonist to differing extents. Thus using ACh as the agonist gallamine always reduced the degree of inhibition produced by atropine (Table 2 & Figure 5) suggesting that gallamine was reducing the affinity of atropine for its binding sites to a greater extent than it affected the affinity of ACh.

It should be noted that Stephenson & Ginsborg (1969) and Ginsborg & Stephenson (1974) have shown that combination of a fast-acting competitive antagonist with a slow-acting competitive antagonist may also lead to a reduction in the dose-ratio rather than an increase under certain conditions. Although gallamine acts more rapidly than atropine this does not appear to be the explanation in these experiments since Stephenson & Ginsborg (1969) found that the effect was only manifested when the agonist occupied a large proportion of the available receptors, that is, it functioned as a partial agonist. However, the experiments reported here with gallamine were conducted using ACh, a full agonist.

In the case of CCh, using gallamine and atropine in combination, the results (Table 3) indicate that gallamine reduces the affinity of both CCh and atropine to a similar extent. Although the combination dose-ratio is close to that predicted for two competitive antagonists, gallamine could also be acting as a metaffinoid antagonist which is equieffective against both CCh and atropine.

Previous workers such as Riker & Wescoe (1951) and Brown & Crout (1968) have concluded that gallamine has an atropine-like action on the heart because it inhibits negative inotropic and chronotropic responses to cholinomimetics in this tissue. Rathbun & Hamilton (1970), in a more extensive investigation, also concluded that gallamine was a competitive antagonist at muscarinic receptors for three reasons. Firstly, gallamine produced parallel rightward shifts of the dose-response curve to the cholinomimetic, with no depression of the maximum response. Secondly, Lineweaver-Burk plots of the effect of gallamine suggested that the antagonism was competitive. However, since Lineweaver-Burk plots involve the use of reciprocal values, they are not ideally suited for distinguishing between competitive and metaffinoid antagonists at high concentrations. Thirdly, Rathbun & Hamilton (1970) found that in pithed rats and spinal cats, combination of gallamine with atropine yielded a combined shift of the log dose-response curve to ACh which was closer to that expected for combination of two competitive antagonists, than for combination of atropine with a non-competitive (metactoid) antagonist. Thus, as there were only two possibilities considered, they concluded that gallamine was a competitive antagonist. However, examination of their data shows there was a less than expected effect from combination of gallamine and atropine with ACh as the agonist, which is in agreement with the findings in guinea-pig atria.

A number of bisquaternary compounds have been reported to possess antimuscarinic activity in a variety of tissues and many of these compounds have also been shown to possess properties different from those of competitive antagonists. These include toxogonin, TMB-4 (Kuhnen-Clausen, 1970; 1972; 1974), hemicholinium-3 (Bieger, Lüllmann & Wassermann, 1968; Mitchelson, 1971; Madden & Mitchelson, 1975) and a series of bisquaternary compounds related to the methonium series (Lüllmann, Ohnesorge, Schauwecker & Wassermann, 1965).

It has also been suggested that the interaction of gallamine with cholinesterases involves an allosteric effect (Changeaux, 1966; Roufogalis & Quist, 1972). Gallamine is a potent inhibitor of cholinesterase in solutions of low ionic strength and Changeaux (1966) found that inhibition of the hydrolysis of ACh by gallamine reached a limiting value, a finding which is analogous to our results on the muscarinic receptors in the atria. More recently, Roufogalis, Quist & Wickson (1973) have suggested that gallamine stabilizes one of two reversible configurations of acetylcholinesterase and that this can account for its effects on enzyme activity. A further point of interest concerning the effects of gallamine on acetylcholinesterase is that gallamine will antagonize the anticholinesterase activity of atropine when ACh is the substrate (Kato, 1971) an effect which bears a similarity to the results on atrial muscarinic receptors, with combination of gallamine and atropine using acetylcholine as the agonist.

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