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Modification of the Binding Properties of Muscarinic Receptors by Gallamine

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SUMMARY

The interaction of gallamine with muscarinic receptors from different tissues has been investigated. Gallamine binds to a site distinct from the conventional muscarinic ligand binding site and modulates the binding of agonists and antagonists to the conventional binding site. In agreement with reported pharmacological studies, the effects of gallamine on the binding of muscarinic ligands are much greater in heart than in other tissues. These findings suggest the possibility of developing novel and selective muscarinic drugs.

INTRODUCTION

The neuromuscular blocking agent, gallamine, has long been known to antagonize the negative inotropic and chronotropic actions of muscarinic agonists in heart, at doses comparable to those producing neuromuscular blockade (1, 2).

Clark and Mitchelson (3) showed that this action is due to an effect of gallamine on muscarinic receptors, but that its mechanism of action is not competitive. In addition, gallamine appears to have a selective effect on heart muscarinic receptors and is a poor antagonist at other muscarinic sites. At doses sufficient to antagonize muscarinic responses in heart, gallamine has no effect on responses in ileum, bladder, or salivary glands (1, 4, 5).

The nature of the interaction of gallamine with muscarinic receptors has been further investigated by examining the effects of gallamine on the binding of muscarinic agonists and antagonists to receptors in rat tissue homogenates. A preliminary report of some of these findings has been published (6).

MATERIALS AND METHODS

[^{3}H]NMS[1] (10 or 53 Ci/mmole) was synthesized as previously described (7) or obtained from New England Nuclear Corporation (Boston, Mass.). Gallamine was purchased from Sigma Chemical Company (St. Louis, Mo.); pirenzepine and 4-diphenylacetoxy-*N*-methylpiperidine methiodide were kind gifts of Dr. Karl Thomae (GmbH) and Dr. R. B. Barlow (University of Bristol), respectively.

Peripheral tissues were homogenized (Polytron, setting 5, two 15-sec treatments, followed by Potter-Elvehjem homogenizer, 520 rpm, 10 up-and-down strokes) in 9 volumes of 20 mM sodium 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid containing 100 mM NaCl and 10 mM $MgCl_2$ (pH 7.0). The homogenization of brain regions was similar, except that the Polytron step was omitted. Homogenates were filtered through one layer of cheesecloth and diluted in the same buffer to give an appropriate protein concentration (0.1–1 mg/ml) which minimized depletion of free [^{3}H]NMS caused by binding to the receptor.

For the binding assays, homogenates were preincubated for 10 min at 30° before addition to microcentrifuge tubes containing an appropriate concentration of [^{3}H]NMS together with unlabeled ligands as indicated in the text or figure legends. Incubation was carried out for 3 hr at 30°, except where indicated in the text, and was terminated by centrifugation at 14,000 × *g* for 2 min at room temperature. Pellets were superficially washed twice in isotonic saline and dissolved in Soluene (Packard). The bound radioactivity was determined by liquid scintillation spectrometry. Assays were routinely carried out in quadruplicate, and nonspecific binding was defined as the radioactivity bound in the presence of 10^{-6} M 3-quinuclidinyl benzilate.

Solutions of gallamine which had been incubated at 30° with heart homogenate for 15 min and for 3 hr produced identical effects on the binding of [^{3}H]NMS to fresh heart homogenate, indicating that gallamine is not chemically altered during long incubations.

Protein was estimated by the method of Lowry *et al.* (8), with bovine serum albumin as standard.

Theoretical binding curves were fitted to the experimental data points by nonlinear least-squares regression analysis (7).

RESULTS

Effect of gallamine on the binding of [^{3}H]NMS in rat heart and cerebral cortex. The classical muscarinic antagonist [^{3}H]NMS binds to both rat heart and cerebral cortical membranes in a buffer containing 100 mM NaCl and 10 mM $MgCl_2$, according to the simple Langmuir isotherm, and with affinity constants of 1.1 to 1.6 × 10^9 M^{-1} (9, 10) and 2.8 × 10^9 M^{-1}, respectively (11). Figure 1 shows the binding of [^{3}H]NMS to rat heart and cerebral cortical homogenates in the presence of varying concentrations of gallamine. Increasing concentrations of gallamine cause a rightward shift of the binding curves resulting from a decrease in [^{3}H]NMS affinity. All of the curves obey the simple Langmuir isotherm (Hill plots of the data have gradients close to 1.0), and nonlinear least-squares regression analysis and Scatchard plots of the data indicate that gallamine does not change the maximal

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[1] The abbreviation used is: NMS, *N*-methylscopolamine.

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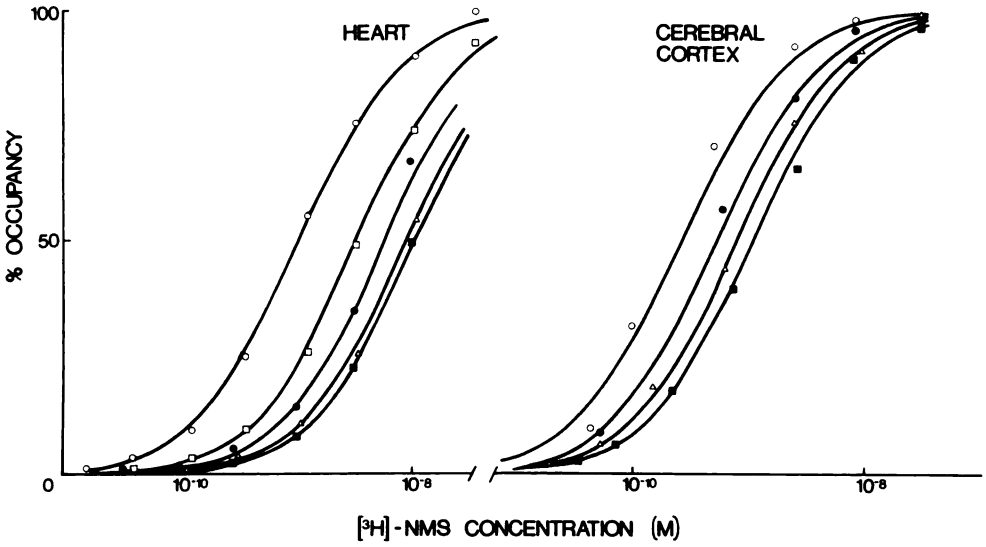


FIG. 1. *Binding of [3H]NMS to rat heart and cerebral cortex in the presence of [^{3}H]NMS*
Binding was determined in the presence of the following concentrations of gallamine: ○, none; □, 3×10^{-6} M; ●, 10^{-5} M; △, 3×10^{-5} M; ■, 10^{-4} M. The curves are best-fit mass-action curves through the experimental points.

number of [^{3}H]NMS binding sites (Fig. 2) even though, in heart, the relatively low ratio of specific to nonspecific [^{3}H]NMS binding prevented measurement of the entire saturation curves at the highest gallamine concentration. However, the interaction between gallamine and [^{3}H]NMS is not competitive since the magnitude of the [^{3}H]NMS affinity shift induced by gallamine is not linear with gallamine concentration, but approaches a limiting value.

A plot of the log (affinity shift − 1) versus log (gallamine concentration in heart), as depicted in Fig. 3, shows that the shift in [^{3}H]NMS affinity approaches a limiting value of approximately 13 at a gallamine concentration of 10^{-4}–10^{-3} M. (The effect in cerebral cortex is much smaller—10^{-4} M gallamine produces only a 4-fold shift in [^{3}H]NMS affinity.) The *dashed line* indicates the theoretical curve for a competitive interaction of two ligands, which clearly does not accord with the experimental findings.

The "noncompetitive" nature of the interaction of gallamine with [^{3}H]NMS is also observed when the binding of a fixed concentration of [^{3}H]NMS is determined in the presence of varying concentrations of gallamine. [^{3}H]NMS binding is increasingly inhibited as the gallamine concentration is increased and reaches a limiting value which is dependent on the NMS concentration (Fig. 4).

A simple reaction which can account for this behavior is shown in Scheme 1. Gallamine (G) and NMS (D) can bind simultaneously to form a ternary complex. The binding of gallamine produces a change in the receptor such that the affinity for NMS is reduced, and vice versa. The equation for the variation of the experimentally determined NMS affinity constant [$K_D(G)$] with gallamine concentration ([G]) is

$$K_D(G) = K_D \frac{(1 + K_2[G])}{(1 + K_1[G]} \qquad (1)$$

The experimental data from Fig. 1 were fitted to Eq. 1, and values for the parameters were determined as

Heart		Cortex	
K_1	= 1.3 (±0.2) × 10^6 M^{-1}	K_1	= 7.5 (±1.1) × 10^4 M^{-1}
K_2	= 9.2 (±2.6) × 10^4 M^{-1}	K_2	= 1.1 (±0.3) × 10^4 M^{-1}
K_D	= 1.2 (±0.2) × 10^9 M^{-1}	K_D	= 3.1 (±0.1) × 10^9 M^{-1}
K_3	= 9.0 (±3.4) × 10^7 M^{-1}	K_3	= 4.6 (±1.4) × 10^8 M^{-1}
K_1/K_2 = 13.4 (±4.6)		K_1/K_2 = 6.8 (±2.1)	

Thus the binding constant of gallamine in the absence of NMS is 16-fold weaker in cerebral cortex than in heart, and the ratio of K_1/K_2, which gives the negative cooperativity of the interaction, also appears to be lower in cerebral cortex than in heart.

The equation for the inhibition (I) of [^{3}H]NMS ([D]) by a given concentration of gallamine ([G]) can also be derived from the scheme, and is as follows:

$$I = \frac{[G](K_1 - K_2)}{1 + K_D[D] + [G](K_1 + K_D K_2[D])} \qquad (2)$$

The experimental data from Fig. 4 for I, [G], and [D] were fitted to this equation and gave independent estimates for the parameters K_1, K_2, and K_D for heart in good agreement with those obtained from Eq. 1 [K_1 = 1.4 (±0.1) × 10^6 M^{-1}; K_2 = 8.9 (±0.9) × 10^4 M^{-1}; K_D = 1.0 (±0.2) × 10^9 M^{-1}; K_1/K_2 = 15.8 (±1.9)]. The *dashed lines* in Fig. 4 indicate the fits to the experimental data.

At concentrations of gallamine greater than 10^{-4} M, deviations from Scheme 1 are observed in which the occupancy of [^{3}H]NMS begins to increase. It is possible, therefore, that there is a second gallamine effect which has not yet been explored in detail.

Effect of gallamine on the binding of [^{3}H]NMS in other tissues from rat. A simple method for evaluating the effect of gallamine on the binding of [^{3}H]NMS to muscarinic receptors in some other tissues was devised. The binding of a tracer concentration of [^{3}H]NMS (10^{-10} M) to tissue homogenates was determined in the presence

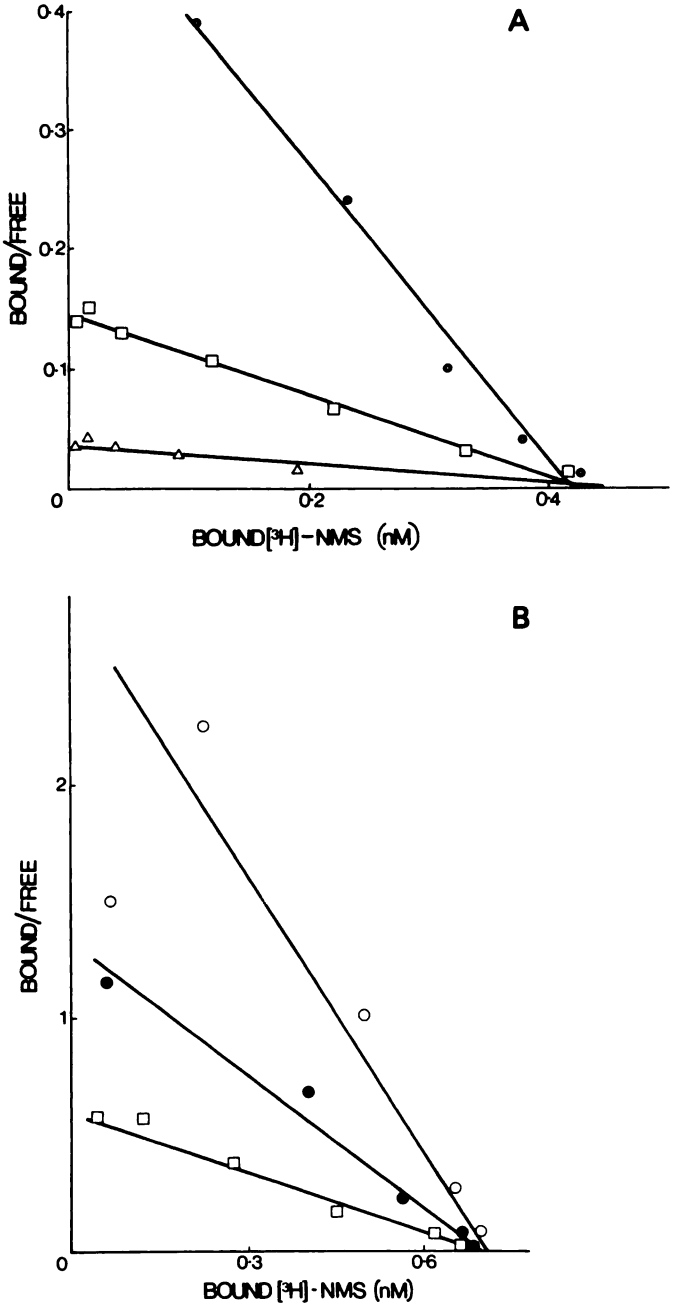


FIG. 2. *Scatchard plots of [³H]NMS binding to gallamine in heart (A) and cerebral cortex (B)*

The plots were drawn from the data in Fig. 1 for the binding of [³H]NMS in the presence of the following concentrations of gallamine: ○, none; □, 3×10^{-6} M; ●, 10^{-5} M; △, 3×10^{-5} M; ■, 10^{-4} M. Nonlinear least squares regression analysis of the data gave values for the NMS affinity constants (K_A) and maximal number of NMS binding sites (B_{max}) as follows:

	Gallamine concentration	K_A (M^{-1})	B_{max} (nM)
Heart	0	$1.2 (\pm 0.4) \times 10^9$	0.42 (±0.028)
	3×10^{-6} M	$3.0 (\pm 0.6) \times 10^8$	0.45 (±0.028) (NS)
	3×10^{-5} M	$1.1 (\pm 0.3) \times 10^8$	0.46 (±0.107) (NS)
Cerebral cortex	0	$3.9 (\pm 2) \times 10^9$	0.71 (±0.093)
	10^{-5} M	$1.9 (\pm 0.8) \times 10^9$	0.70 (±0.048) (NS)
	10^{-4} M	$8.6 (\pm 0.3) \times 10^8$	0.69 (±0.064) (NS)

Figures in parentheses indicate 95% confidence limits. NS, Not significantly different from B_{max} in the absence of gallamine.

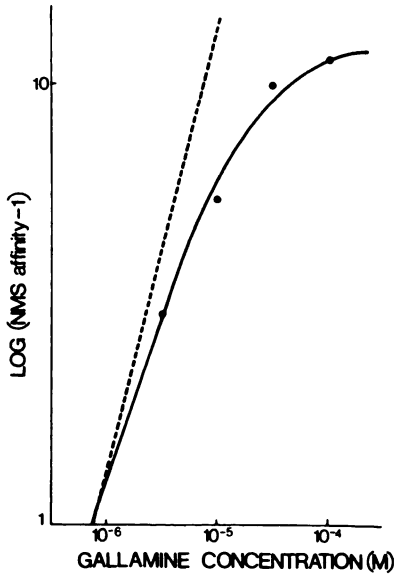


FIG. 3. *Gallamine-induced shift in [³H]NMS affinity in rat heart*

The [³H]NMS affinity constants were derived from the data in Fig. 1. The *dashed line* represents the theoretical curve for the competitive interaction between NMS and a drug with affinity constant 1.3×10^8 M^{-1}. The *solid line* represents the nonlinear least-squares fit of the data to Eq. 1 in the text.

of a range of gallamine concentrations. At this concentration, NMS has a very low occupancy and its effect on the binding of gallamine is very small. Therefore, the concentration of gallamine producing half-maximal reduction of [³H]NMS binding is a good estimate of the dissociation constant of gallamine (K_1). In addition, the maximal reduction of [³H]NMS binding produced by gallamine is a good estimate of the maximal shift of NMS affinity that can be induced by gallamine (K_1/K_2). The results obtained for a variety of tissues show that gallamine has a greater effect on heart than on any other tissue, in terms of both its binding constant and the maximal induced shift of NMS affinity (Table 1). Thus,

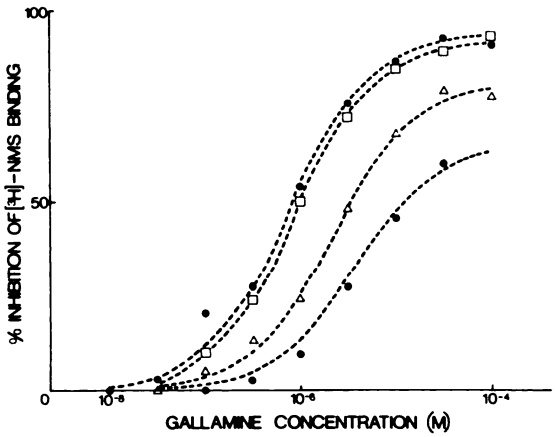
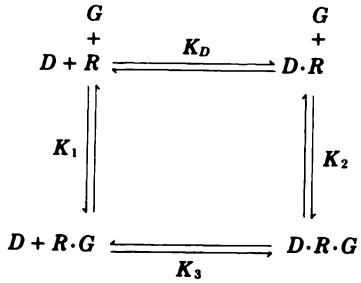


FIG. 4. *Gallamine inhibition of [³H]NMS binding in rat heart*

The inhibition by gallamine of the binding of the following concentrations of [³H]NMS was determined: ○, 2.5×10^{-11} M; □, 2.3×10^{-10} M; △, 2.4×10^{-9} M; ●, 6.8×10^{-9} M. The *dashed lines* represent the theoretical curves for the interaction of two ligands according to the scheme of negative heterotropic cooperativity given in Eq. 2 and using the parameters stated in the text.



SCHEME 1

Scheme for the interaction of gallamine and a muscarinic drug with the muscarinic receptor

D, Muscarinic drug; *G*, gallamine; *R*, muscarinic receptor. K_1 is the binding constant of *G* in the absence of *D*, and K_2 is the binding constant of *G* when the receptor is saturated with *D*.

at 10^{-6} M gallamine, the affinity of NMS in heart is halved, whereas that in submandibular gland is reduced by only 2%.

Effect of gallamine on the binding of other muscarinic antagonists. In order to determine the effect of gallamine on the binding of nonradiolabeled antagonists, it is necessary to determine the effect of gallamine on the ability of these drugs to compete with a labeled antagonist. The interaction can be described by Scheme 2. *L* and *D* compete for the same binding site, and *G* does not compete with either *L* or *D*. If the effect of increasing concentrations of *L* on the binding of *D* is determined in the absence of *G*, the concentration of *L* giving 50% inhibition of *D* (IC_{50}) can be used to calculate K_L as follows:

$$1/K_L = IC_{50}(1 + [D]K_D) \quad (3)$$

The concentration of *L* giving 50% inhibition of the binding of *D* in the presence of *G* (IC_{50} [*G*]) can then be related to the scheme parameters as follows:

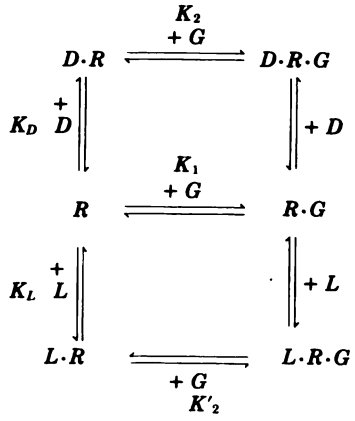
$$1/IC_{50}(G) = \frac{K_L(1 + K'_2[G]}{1 + K_1[G] + K_D[D](1 + K_2[G]} \quad (4)$$

If K_1, K_2, K_D, and K_L are known, we can determine the binding constant of *G* for the *LR* complex, K'_2, and the ratio K_1/K'_2 gives the negative cooperativity of the interaction between gallamine and the unlabeled drug.

TABLE 1

Effect of gallamine on the binding of [^{3}H]NMS (10^{-10} M) in a variety of rat tissue homogenates

Tissue	Gallamine concentration producing half-maximal inhibition of [^{3}H]NMS binding	Maximal decrease in [^{3}H]NMS binding
	M	*-fold*
Heart	8×10^{-7}	13
Ileal smooth muscle	1.3×10^{-5}	2
Lachrymal gland	3×10^{-5}	2
Submandibular gland	2×10^{-5}	<2
Central nervous system		
Cerebral cortex	1.3×10^{-5}	6
Hippocampus	1.6×10^{-5}	3
Striatum	2.5×10^{-5}	3
Medulla-pons	3×10^{-6}	3



SCHEME 2

Scheme for the interaction of two competitive muscarinic drugs and gallamine with the muscarinic receptor

D, Radiolabeled muscarinic drug; *L*, unlabeled muscarinic drug; *G*, gallamine; *R*, muscarinic receptor.

This method was used to indicate the values for the negative cooperativity of the interaction of a wide range of antagonists with gallamine, in both heart and cerebral cortex. The effect of increasing concentrations of these drugs on the binding of 3×10^{-10} M [^{3}H]NMS was determined in the absence and presence of either 10^{-4} M or 10^{-3} M gallamine. The values of negative cooperativity obtained are shown in Table 2. They are not the same for all drugs, as would be the case if the interaction was competitive. In general, the effect of gallamine on the binding of most drugs is greater in heart than in cerebral cortex, but the rank order of negative cooperativity for the drugs is different in the two tissues.

Most of these determinations were made using a gal-

TABLE 2

Estimates of the negative cooperativity of binding to muscarinic receptors between gallamine and some muscarinic antagonists in rat heart and cerebral cortex

Drug	Negative cooperativity	
	Heart	Cerebral cortex
	-fold	
Using 10^{-3} M gallamine		
Di-4[a]	8	8
(−)-Scopolamine	13	18
(−)-NMS	14	8
(±)-*N*-methylatropine	16	9
(±)-Atropine	28	15
Benzhexol	40	24
Propylbenzilylcholine	46	15
Clozapine	78	40
Pirenzepine[b]	88	77
Cyproheptidine	91	53
Using 10^{-4} M gallamine		
(−)-NMS	13	—
(±)-Atropine	31	—
Propylbenzilylcholine	45	—

[a] 4-Diphenylacetoxy-*N*-methylpiperidine methiodide.

[b] Non-mass action binding in cerebral cortex was found, indicating binding to more than one site. Gallamine shifts the binding curve in a nonparallel fashion.

lamine concentration of 10^{-3} M, a concentration at which, as we have already mentioned, some deviations from Scheme 1 occur. It was necessary to use this high concentration, because the sensitivity of the method is limited by the values of $K'_2 \cdot [G]$ and $K_1 \cdot [G]$, with larger values giving greater sensitivity. In cerebral cortex, the lowest gallamine concentration at which the interaction of drugs with gallamine can be reliably distinguished from competitive behavior by this method is 10^{-3} M. In heart, 10^{-4} M gallamine can be used. We are confident, however, that the values in Table 2 are good approximations of the negative cooperativities of the drugs, since the deviations which occur from Scheme 1 at high gallamine concentrations are apparent only at concentrations of [^{3}H]NMS greater than 3×10^{-10} M. Where determinations have been made using both 10^{-3} M and 10^{-4} M gallamine, no significant difference was observed in the values of negative cooperativity obtained.

TABLE 3

Estimates of the negative cooperativity of binding to muscarinic receptors between gallamine and some muscarinic agonists in heart and cerebral cortex

Drug	Negative cooperativity	
	Heart	Cerebral cortex
	-fold	
Butyrylcholine	41	21
Aceclidine	70	26
Carbachol	78	90
Acetylcholine	100	67
Pilocarpine	110	24
Oxotremorine	120	90
Tetramethylammonium	120	37
Methylfurmethide	145	130
Arecoline	170	90
Oxotremorine-M	250	100

Effect of gallamine on the binding of muscarinic agonists. Similar experiments were performed for a range of muscarinic agonists, and estimates of their negative cooperativities are shown in Table 3. The effects on agonist binding were generally found to be larger than on antagonist binding, but the figures have to be taken as only indicative, since muscarinic agonists bind to a heterogeneous population of binding sites, termed *L*, *H*, and *SH* sites (12, 13).

A more detailed analysis of the effects of gallamine on carbachol binding indicates that the effect on each population of carbachol binding sites is not the same. Carbachol binding curves in heart and cerebral cortex are shifted by gallamine in a nonparallel fashion (Fig. 5). In heart, the lower-affinity end of the curve shows the largest shift, whereas in cerebral cortex the higher-affinity end of the curve is shifted most.

The curves were fitted to a three-site model using a program of nonlinear least-squares regression analysis (7), and values for the binding constant of carbachol at each site in the absence and presence of gallamine were obtained (Table 4).

Effect of gallamine on the kinetics of [^{3}H]*NMS binding to muscarinic receptors.* The binding of [^{3}H]NMS to tissue homogenates in the absence of any other drug, and under the experimental conditions described here (30°, pH 7.0), reaches equilibrium within 5 min. During preliminary experiments we observed that a considerably longer incubation time was necessary for [^{3}H]NMS binding to reach equilibrium if gallamine was present in the incubation medium. Investigation of the kinetics of [^{3}H]NMS binding showed that the association of NMS with time involves a very rapid phase followed by a much slower

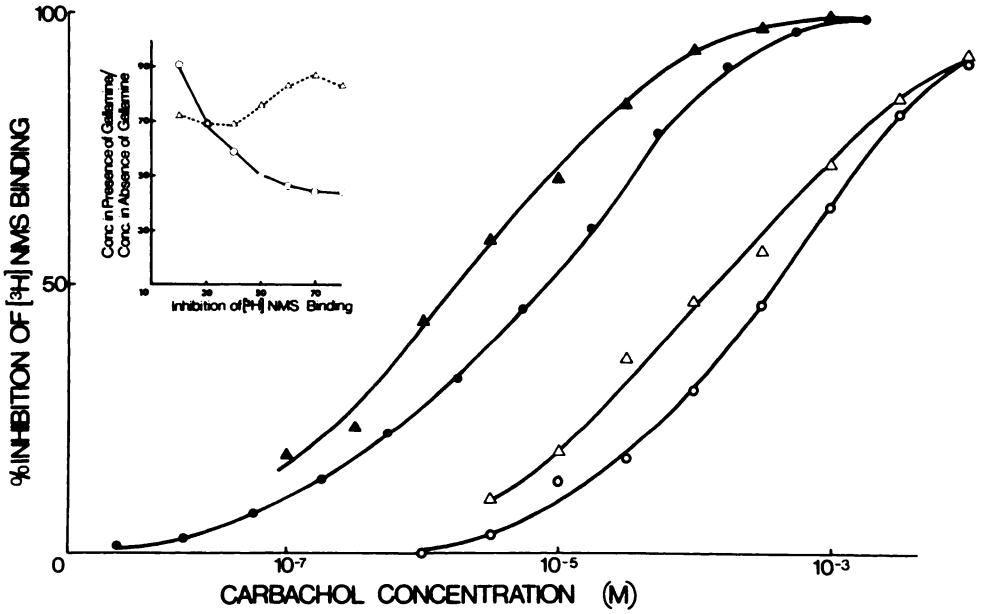


FIG. 5. *Effect of gallamine on the binding of carbachol to muscarinic receptors*

Carbachol inhibition of the binding of 3×10^{-10} M [^{3}H]NMS, in the absence (*solid symbols*) and presence (*open symbols*) of 10^{-3} M gallamine, in heart (▲, △) and cerebral cortex (●, ○). The *inset* is a plot of the fold increase in carbachol concentrations needed to produce the same inhibition of [^{3}H]NMS binding in the presence of gallamine as in its absence, for increasing percentage inhibition in heart (△) and cerebral cortex (○).

TABLE 4

Effect of gallamine on the affinity constants (K) of carbachol for binding to the L, H, and SH affinity sites in heart and cerebral cortex

The inhibition of [^{3}H]NMS binding by increasing concentrations of carbachol was determined in the absence and presence of 10^{-3} M gallamine. Data (shown in Fig. 5) was fitted to a three-site model using a program of nonlinear least-square regression analysis, and values of log *K* (with standard errors) for each site were obtained.

Tissue	Ligand	Site	Log $K_{control}$	Log *K*+ gallamine	Ratio
Cerebral cortex	Carbachol	*L* (55%)	4.40 ± 0.05	2.67 ± 0.12	59
		H (30%)	5.78 ± 0.11	3.70 ± 0.13	120
		—*SH* (15%)	7.14 ± 0.20	ND[a]	—
Heart	Carbachol	*L* (26%)	4.44 ± 0.18	2.34 ± 0.38	126
		H (52%)	5.77 ± 0.10	3.93 ± 0.20	70
		—*SH* (22%)	7.19 ± 0.30	5.43 ± 0.51	60

[a] ND, Not determined: a three-site fit, including the minor population of —SH sites, did not converge.

phase, as has been reported for other muscarinic antagonists (14, 15). Gallamine decreased the association rate of NMS in a dose-dependent manner, both by decreasing the proportion of the rapidly associating component and by decreasing the rate of the slower phase (Fig. 6A). The effect does not appear to have reached a limiting value with a gallamine concentration of 10^{-3} M.

The dissociation rate of NMS also has a complex relationship with time, and gallamine decreased this also, again by effects on both the rapid and slow phases (Fig. 6B). However, the extent of the decrease was not as great as that for the association rate.

Because of the considerable effect of gallamine on the kinetics of [^{3}H]NMS binding, it was found to be necessary to incubate homogenates with the ligands for 3 hr in experiments involving gallamine, to ensure that equilibrium was reached (with concentrations of gallamine above 10^{-3} M, equilibrium was not reached even after this time). The long incubation time did not appear to affect the other binding properties of the receptors, or to produce any chemical modification of gallamine.

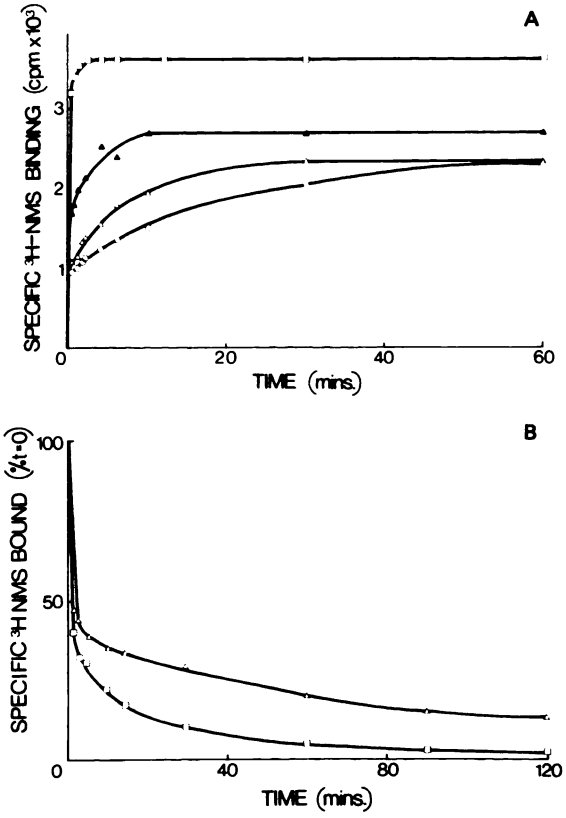


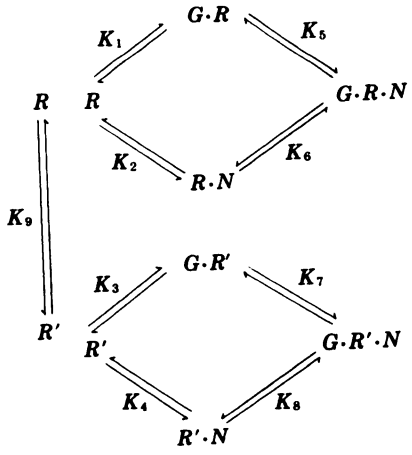
FIG. 6. *Effect of gallamine on association (A) and dissociation (B) rates of [^{3}H]NMS binding*

A. [^{3}H]NMS (3×10^{-9} M) was added to tissue homogenates containing the following concentrations of gallamine: □, none; ▲, 5×10^{-5} M; △, 3×10^{-4} M; ○, 1×10^{-3} M. Samples were incubated at 30° and the specific [^{3}H]NMS bound was determined at varying time intervals.

B. [^{3}H]NMS (10^{-8} M) was equilibrated with tissue homogenates for 2 hr at 30°. Unlabeled NMS (10^{-6} M) was then added ($t = 0$) in the absence (□) or presence (△) of 3×10^{-4} M gallamine, and the specific [^{3}H]NMS bound was determined at varying time intervals.

DISCUSSION

The actions of gallamine described in this paper cannot be explained by the binding of gallamine at the same site that binds muscarinic antagonists or agonists. They can be explained by the binding of gallamine to a second binding site, which causes a change in the properties of the site that binds the agonists and antagonists. This change is most easily understood in terms of an altered conformation state of the receptor. A simple reaction is shown in Scheme 3 in which the receptor is postulated to exist in two conformations, *R* and *R'*, which have different affinities for both NMS and gallamine. Either ligand will therefore change the proportions of *R* and *R'* and hence alter the binding of the other. This type of model has been discussed previously by Birdsall *et al.* (16) and Burgen (17), and an allosteric mechanism has recently



SCHEME 3

Scheme for the interaction of gallamine and NMS with the muscarinic receptor which exists in two conformations

N, NMS; *G*, gallamine; *R* and *R'*, two preexisting conformations of the muscarinic receptor.

Assumptions: $K_1 = K_6$, $K_2 = K_5$, $K_3 = K_8$, $K_4 = K_7$.

Equations:

(a) $$K_G(N{=}O) = \frac{K_1 + K_3 \cdot K_9}{1 + K_9}$$

(b) $$K_N(G{=}O) = \frac{K_2 + K_4 \cdot K_9}{1 + K_9}$$

(c) $$K_{coop} = \frac{(K_1 \cdot K_2 + K_3 \cdot K_4 \cdot K_9)(1 + K_9)}{(K_2 + K_4 \cdot K_9)(K_1 + K_3 \cdot K_9)}$$

been invoked to explain the interaction of enkephalins and morphine at the opiate receptor (18). It is analogous to allostery in enzymology (19), and has also been called metaffinoid antagonism (20).

The model can account for the inverse correlation of gallamine affinity and NMS affinity and can also account for differing degrees of negative cooperativity in different tissues. It could account for the complex kinetics of NMS association and dissociation with the receptor if the conversion of the conformational forms could only occur for the free, unliganded receptor. As with all models, the minimal complexity should be postulated to account for the phenomena observed, and in this sense the model of Scheme 3 is a minimal model.

Ben Baruch *et al.* (21) have also recently suggested the existence of a drug binding site on muscarinic receptors distinct from the antagonist binding site. Their studies on the interaction of the antiestrogenic drug clomiphene citrate with muscarinic receptors indicate the binding of more than one molecule of clomiphene. It is possible, therefore, that this drug is binding to the "gallamine binding site" in addition to the classical antagonist binding site.

It is not certain whether the gallamine and antagonist binding sites are on the same protein subunit, but the gallamine effect persists in solubilized receptor in which the antagonist binding site is apparently integral to a monomeric protein unit of molecular weight ~80,000.[2] This would appear to distinguish it from the effects of guanine nucleotides, where the effector is probably on a separate protein subunit.

Gallamine binding to nicotinic receptors is reported to be essentially competitive in character (22), but it is interesting that it is a noncompetitive inhibitor of acetylcholinesterase, where it appears to act by an allosteric mechanism similar to that discussed in this paper for muscarinic receptors (23). Changeux (23) has discussed the interesting possibility that, because of the structural resemblance of gallamine to lecithin, the gallamine binding site on acetylcholinesterase might be physiologically concerned with the binding of phospholipids in the membrane. This might also apply to the muscarinic receptor.

The results from binding studies correlate well with the behavior of gallamine in pharmacological studies on guinea pig atria described by Clark and Mitchelson (3). In addition, our results showing that the effects of gallamine on the binding of muscarinic ligands are much greater in heart than in other tissues are in good agreement with the described pharmacological cardioselectivity of gallamine (3–5). The finding that responses to muscarinic agonists can be altered by the binding of drugs to a completely different site provides the possibility of developing muscarinic drugs with novel structures and tissue selectivity.

The mechanism of action of gallamine described here conflicts with a recent report that gallamine interacts competitively with brain muscarinic receptors (24). The interaction was described as competitive on the basis that gallamine did not change the maximal number of [^{3}H]quinuclidinyl benzilate binding sites (B_{max}). However, we have demonstrated that a drug cannot be assumed to be competitive solely because it does not change the B_{max} (Figs. 2 and 3). The possibility exists that many muscarinic drugs that were thought to be competitive may be interacting with the "gallamine site" rather than the "acetylcholine site," since if the negative cooperativity is very large it may not be technically feasible to distinguish between the two modes of binding. Caution should therefore be exercised when assigning the terms "competitive" and "noncompetitive" to the interaction of a drug with muscarinic receptors, and it should be borne in mind that a structure-activity series may in fact be a composite of two such series relevant to two distinct binding sites.

[2] E. C. Hulme, C. P. Berrie, J. M. Stockton, N. J. M. Birdsall, and A. S. V. Burgen, unpublished results.

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