

AD-A274 877



REPORT DOCUMENTATION PAGE

①

1 AGENCY USE ONLY		2 REPORT DATE 1992		3 TYPE/DATES COVERED	
4 TITLE AND SUBTITLE A PHARMACOLOGICAL PROFILE OF GLYCOPYRROLATE: INTERACTIONS AT THE MUSCARINIC ACETYLCHOLINE RECEPTOR				5 FUNDING NUMBERS	
6 AUTHOR WAI-MAN LAU AND MARIA SZILAGYI					
7 FORMING ORG NAMES/ADDRESSES DEFENCE SCIENCE AND TECHNOLOGY ORG. MATERIALS RESEARCH LABORATORY, P O BOX 50 ASCOT VALE, VICTORIA 3032, AUSTRALIA				8 PERFORMING ORG. REPORT NO	
9 SPONSORING/MONITORING AGENCY NAMES AND ADDRESSES					
11 SUPPLEMENTARY NOTES					
12 DISTRIBUTION/AVAILABILITY STATEMENT DISTRIBUTION STATEMENT A				12B DISTRIBUTION CODE	
13. ABSTRACT (MAX 200 WORDS): GLYCOPRROLATE, A SYNTHETIC QUATERNARY AMMONIUM COMPOUND HAD BIPHASIC EFFECTS ON THE CONTRACTION OF GUINEA-PIG ATRIUM. AT CONCENTRATIONS BETWEEN 0.4 AND 20UM, GLYCOPYRROLATE INDUCED A SMALL BUT CONSISTENT INCREASE IN THE CONTRACTION FORCE. OUR RESULTS CONFIRM THE ANTIMUSCARINIC PROPERTIES OF GLYCOPRROLATE. WE SUGGEST THAT THE THERAPEUTIC VALUE OF GLYCOPYRROLATE IN TREATMENT OF ORGANOPHOSPHATE POISONING IS ASSOCIATED WITH ITS POTENCY AGAINST PROLONGED MUSCARINIC RECEPTOR ACTIVATION BY CHOLINERGIC AGONISTS.					
14 SUBJECT TERMS				15 NUMBER OF PAGES 6	
				16 PRICE CODE	
17 SECURITY CLASS.REPORT	18 SEC CLASS PAGE UNCLASSIFIED	19 SEC CLASS ABST.	20 LIMITATION OF ABSTRACT		

DISTRIBUTION STATEMENT A
Approved for public release

DISTRIBUTION STATEMENT A
Approved for public release
Distribution Unlimited

DTIC
SELECTED
JAN 19 1994
S B D

A PHARMACOLOGICAL PROFILE OF GLYCOPYRROLATE: INTERACTIONS AT THE MUSCARINIC ACETYLCHOLINE RECEPTOR

WAI-MAN LAU* and MARIA SZILAGYI

Materials Research Laboratory, Defence Science and Technology Organisation, P.O. Box 50, Ascot Vale,
Victoria 3032, Australia

(Received 9 March 1992)

Abstract—1. Glycopyrrolate, a synthetic quaternary ammonium compound had biphasic effects on the contraction of guinea-pig atrium. At concentrations between 0.4 and 20 μ M, glycopyrrolate induced a small but consistent increase in the contraction force.

2. Further increase in the concentration of glycopyrrolate produced a concentration-dependent reduction in the force of contraction with a EC_{50} of 0.24 mM.

3. This negative inotropic effect was opposed by a Ca^{2+} channel agonist, Bay K 8644. Glycopyrrolate also antagonized potently the depressant effects of carbachol and acetylcholine in guinea-pig atrium.

4. Schild analysis showed a pA_2 value of 8.16 against carbachol and 8.39 against acetylcholine. These similar pA_2 values suggested that both compounds may have a common interacting site. The interactions however cannot be explained by a simple competition model as the slopes of the Schild plots were larger than unity.

5. The mutual competition between glycopyrrolate and quinuclidinyl benzilate (QNB), gallamine or methoctramine indicated that glycopyrrolate could have multiple action sites in the atrium. Interaction at an allosteric site was implicated.

6. Radioligand binding studies showed that glycopyrrolate displaced pirenzepine (PZ) and AF-DX 384 from their binding to the M_1 and M_2 muscarinic receptors in guinea-pig brain membranes respectively.

7. The respective binding constants (K_d s) were 0.60 and 0.03 nM. The Hill coefficient value (n_H) for glycopyrrolate against [3H] PZ was larger than unity, suggesting positive cooperativity at the receptor complex. In contrast, the n_H of the agonist [3H] AF-DX 384 was not different from unity, indicating a simple competitive inhibition.

8. Our results confirm the antimuscarinic properties of glycopyrrolate. We suggest that the therapeutic value of glycopyrrolate in the treatment of organophosphate poisoning is associated with its potency against prolonged muscarinic receptor activation by cholinergic agonists.

INTRODUCTION

Glycopyrrolate is a synthetic quaternary ammonium compound, known to have antimuscarinic effects. Together with atropine, glycopyrrolate is commonly used during anaesthesia as an antisialagogue and to prolong the effectiveness of some anticholinesterases such as physostigmine and neostigmine against neuromuscular blockade (Muravchick *et al.*, 1979). This effect is apparently unrelated to the inhibition of acetylcholinesterase as glycopyrrolate is not an acetylcholinesterase inhibitor (Lau and Szilagyi, 1991, data not shown). In contrast, Wali *et al.* (1987) suggested that glycopyrrolate intensifies neuromuscular blockade, delays recovery after atracurium and is a potent antagonist of the muscarinic effects of neostigmine in rat isolated phrenic nerve-diaphragm preparation. Studies by Richards *et al.* (1989) indicated that glycopyrrolate when administered intramuscularly produces less tachycardia than atropine in anaesthetized patients. This may be due to the slow penetration of glycopyrrolate

through the blood-brain barrier (Proakis and Harris, 1979) which results in smaller interferences with the central cholinergic pathways associated with cardiac innervations.

Recently, glycopyrrolate has been used in combination with atropine, benzodiazepine and pralidoxime to treat patients intoxicated by organophosphates. The combined formulations gave superior control of bronchial secretions and minimized the occurrence of bradycardia. Furthermore, the combination therapy produced fewer central toxic symptoms in comparison to cases when larger doses of atropine alone were used (Tracey and Gallagher, 1990). This evidence points to the usefulness of glycopyrrolate in treating medical conditions with cholinergic implications, although its pharmacological properties have not been fully determined.

In the present investigation, we have examined the antagonism by glycopyrrolate of the negative inotropic effects due to acetylcholine and carbachol. Mutual competition studies with muscarinic antagonists and displacement experiments against specific muscarinic radioligands were also conducted to elucidate possible sites of action of glycopyrrolate.

*To whom all correspondence should be addressed.

MATERIALS AND METHODS

Guinea-pig atrial preparations

Left atrial preparations were surgically removed from female guinea-pigs weighing 250–400 g as described by Freeman and Turner (1974). Preparations were transferred to an organ bath containing Ringer's heart solution (composition mM: NaCl 115, KCl 4.6, CaCl₂ 1.8, MgSO₄ 1.2, NaHPO₄ 1.2, NaHCO₃ 22, glucose 22, pH = 7.4) at 37°C. The buffer was continuously aerated with carbogen (95% O₂ and 5% CO₂). The preparations were allowed to equilibrate for 30 min before the experiment commenced. The atria were stimulated at 2 Hz and 10 V and the tension of isometric contraction was recorded by a Shinkoh U-gauge (UL-2-120). The signal outputs were modulated by a Coulbourn transducer (S72-75) and recorded by a Graphtech linear recorder (WR-3071). Tension development was constant during the period of the experiments which lasted for approx. 3 hr.

Brain membrane preparations for receptor binding assays

Female guinea-pigs were killed by cervical dislocation and the brains rapidly removed for membrane preparation. Each guinea-pig brain weighing 2–4 g was dissected into different brain regions, the cortex and cerebellum. The cortex was homogenized in 20 ml ice-cold Tris-HCl (50 mM, pH = 7.7) and the cerebellum in 20 ml ice-cold Na-K phosphate buffer (10 mM, pH = 7.5) by an Ultra-Turrax homogenizer on setting 6 for two 15 sec bursts with 30 sec rest period in between. The homogenate was then centrifuged at 18,800 g for 30 min at 4°C. The pellet was resuspended in 20 ml buffer and recentrifuged under the previous conditions. The final pellet was resuspended in 10 ml buffer. Extracts from cortex were later incubated with [³H] PZ for the assay of M₁ muscarinic receptors, while those from cerebellum were equilibrated with [³H] AF-DX 384 for M₂ subtype assay. Protein concentration of the cortical extracts ranged from 6 to 10 mg/ml, and of the cerebellum extracts, 4–6 mg/ml.

Displacement studies

200 µl of the cortical or cerebellum extracts was incubated with varying concentrations of glycopyrrolate (0.01 nM–0.1 µM) in a total volume of 2 ml. For M₁ receptor assay, 375 pM [³H] PZ was used and for M₂ assay, 220 pM [³H] AF-DX 384 was applied. Non-specific binding for [³H] PZ was defined with 1 µM QNB and for [³H] AF-DX 384, 1 µM atropine.

The incubation time was 2 hr for [³H] PZ and 1 hr for [³H] AF-DX 384. Binding was terminated by filtering through polyethyleneimine (0.05% w/v) moistened Whatman GF/B filter paper using a Brandel Cell Harvester. The filters were rapidly washed with 15 ml cold buffer in five aliquots and equilibrated with 10 ml Beckman Readyprotein scintillant for 24 hr. Radioactivity was measured after equilibration with a Beckman Liquid Scintillation System LS 5801; with a counting efficiency of 50% for tritium.

Protein determination

The protein content of the brain membranes was measured according to the Lowry *et al.* method (1951).

Data analysis

The concentration–response curves were constructed by adding drugs cumulatively to the organ bath. This did not apply to the experiments for ACh, as the atria developed desensitization at higher concentrations of ACh. Consequently, fresh ACh was administered after the previous dose was washed out. Data are presented as mean ± SEM; and the difference between the means was analysed by the Student's *t*-test (Downie and Heath, 1970).

The results of the displacement experiments were analysed by a Hill plot to obtain the Hill coefficient (*n_H*) and the inhibition constant (*K_i*) for glycopyrrolate. The binding

dissociation constant (*K_d*) required for the calculation of *K_i* was determined in other experiments. Details of the method have been described by Freeman *et al.* (1986).

Chemicals

Atropine, gallamine, carbachol and acetylcholine were obtained from Sigma, St Louis, Mo, U.S.A. (±) Quinuclidinyl benzilate (QNB), Bay K 8644 and methoctramine were from Research Biochemicals Incorporated, Natick, U.S.A. Glycopyrrolate was a gift from A. H. Robbins, Sydney. [³H] PZ (87.0 Ci/mmol) and [³H] AF-DX 384 (97.0 Ci/mmol) were purchased from DuPont, Wilmington, U.S.A. All other chemicals used were AR grade and are readily available from the usual commercial sources.

RESULTS

Effects of glycopyrrolate on atrial contractility

Glycopyrrolate at different concentration ranges produced opposite effects on the atrial force of contraction. In the lower concentration range (0.4–20 µM), glycopyrrolate marginally increased the force by 4–13%. In contrast, increasing the concentration from 40 µM to 0.8 mM induced a concentration-dependent reduction in the force of contraction with a EC₅₀ of 0.24 ± 0.03 mM (Fig. 1). At 0.8 mM, glycopyrrolate completely stopped atrial contraction. Addition of 0.5 µM Bay K 8644, a Ca²⁺ channel agonist (Fleckenstein, 1984) antagonised the negative effect of glycopyrrolate by shifting the concentration–response curve to the right (Fig. 1). The EC₅₀ for glycopyrrolate was increased to 0.47 ± 0.03 mM which was significantly different (*P* < 0.05, *n* = 6) from the value obtained in the absence of Bay K 8644. The negative inotropic effect of glycopyrrolate on the left atrium was readily reversible as the force of contraction rapidly returned to the control level when the drug was washed out.

Interactions with muscarinic agonists

Glycopyrrolate (0.01–0.1 µM) potently antagonised the negative inotropic effects induced by acetylcholine and carbachol. The concentration–response curves for both muscarinic agonists were displaced in parallel to the right in a manner dependent on the concentration of glycopyrrolate (Fig. 2). The antagonism was fully surmountable as high concentrations of the muscarinic agonists were capable of attaining

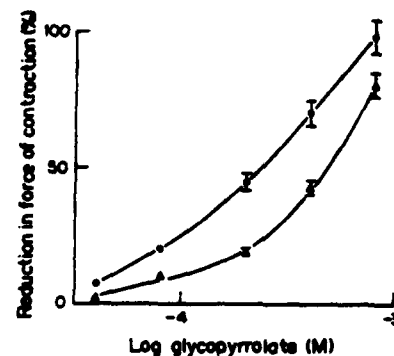


Fig. 1. The negative inotropic effects of glycopyrrolate in the absence (●) and presence (▲) of 0.5 µM Bay K 8644 on guinea-pig atrium. Each point is the mean ± SEM of 6 observations.

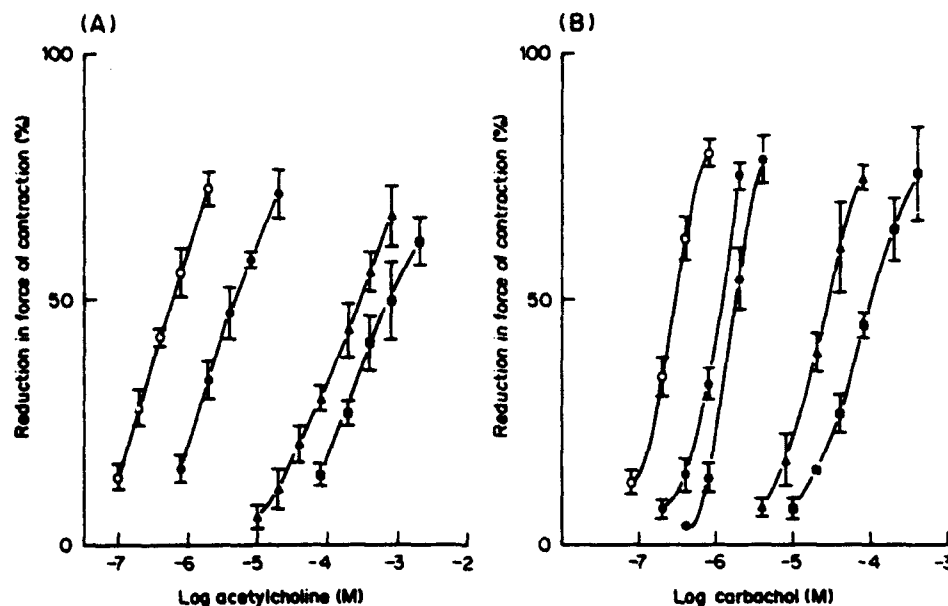


Fig. 2. The effect of glycopyrrolate on the negative inotropic effect of (A) acetylcholine and (B) carbachol. Each point is the mean $\pm$ SEM of 4–6 observations. Controls (○); glycopyrrolate concentration administered: 10 nM (●), 20 nM (◐), 50 nM (▲) and 100 nM (■).

maximum reduction in the arterial force of contraction in the presence of glycopyrrolate. Analysis of the data by Schild plot (Arunlaskhana and Schild, 1959) showed a linear relationship ($r \leq 0.95$). The pA_2 value for glycopyrrolate was 8.16 against carbachol and 8.39 against acetylcholine. The respective slopes of the plots were 2.2 and 3.0 (Fig. 3). They are both significantly different from unity ($P < 0.05$); indicating that the antagonism is not of the simple competitive type.

Antagonism to carbachol by glycopyrrolate and muscarinic antagonists alone or in combination

The antagonism of the negative inotropic effects of carbachol by glycopyrrolate, in combination with the muscarinic antagonists QNB, gallamine and methoc-

tramine was investigated. Based on the method of Paton and Rang (1965), the dose ratios of the experiment in which the two drugs were applied simultaneously should not be different from the combined dose ratios individually determined for the two antagonists if they compete at the same site. If they do not compete, but combination with either drug blocks the receptor, then the experimental dose ratio should be equivalent to the product of the individual dose ratio of the two antagonists. Table 1 shows the dose ratios for glycopyrrolate, QNB, gallamine and methoc-tramine, alone and in combination, competing with the muscarinic agonist carbachol. The experimental dose ratio of glycopyrrolate (50 nM) and QNB (5 nM) was 225. It was significantly ($P < 0.05$) larger than the ratio (37) expected for a combination of two

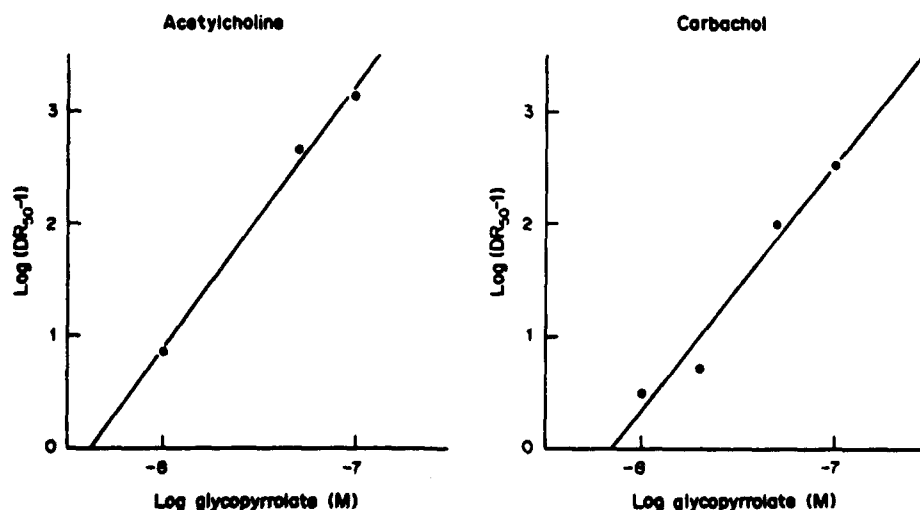


Fig. 3. Schild plot for the antagonism between glycopyrrolate and acetylcholine or carbachol at atrial muscarinic receptors in guinea-pig left atrium. The points are the means of 4–6 experiments.

Table 1. Dose ratios produced by glycopyrrolate in combination with QNB, gallamine or methoctramine on muscarinic receptors of guinea-pig atrium with carbachol as agonist

Glycopyrrolate concentration (nM)	Glycopyrrolate dose ratio (A)	QNB (5 nM) dose ratio (B)	Gallamine (0.1 mM) dose ratio (B)	Methoctramine (1 μ M) dose ratio (B)	Experimental obtained dose ratio	Expected dose ratio (A + B - 1)
50	67.0 $\pm$ 9.8 (n = 10)	21.0 $\pm$ 8.5 (n = 5)			225.0 $\pm$ 8.1* (n = 3)	87.0
50	67.0 $\pm$ 9.8 (n = 10)		81.1 $\pm$ 11.6 (n = 10)		212.2 $\pm$ 34.9 (n = 5)	147.1
50	67.0 $\pm$ 9.8 (n = 10)			68.5 $\pm$ 15.6 (n = 6)	133.8 $\pm$ 56.3 (n = 3)	134.5

The value of the dose ratio is given by mean $\pm$ SEM, n = No. of experiments.

*Difference is significant at $P < 0.05$ level.

competitive antagonists (Table 1). In contrast, a combination of glycopyrrolate (50 nM) and methoctramine (1 μ M), a cardiac M_2 antagonist (Melchiorre *et al.*, 1989) produced a dose ratio of 134 which was not different from the value expected (135) for these two competitive antagonists. The same can be said for glycopyrrolate (50 nM) and gallamine (0.1 mM), a muscarinic allosteric effector (Mitchelson, 1988) which did not show any significant difference between the expected (147) and the experimentally combined dose ratio (212).

Inhibitory actions at the muscarinic acetylcholine receptors

The inhibitory effects of glycopyrrolate on [3 H] PZ, and [3 H] AF-DX 384 were determined by measuring the radioactivity of the ligand-receptor complex in the presence of increasing concentrations of the inhibitor and constant radioligand concentration. The results were analysed by a Hill plot, i.e. $\log [Y/(1-Y)]$ vs $\log I$ where Y is the ratio of the radioligand bound in the presence of glycopyrrolate to that in its absence, and I is the concentration of glycopyrrolate. The slope of the plot is known as the Hill coefficient (n_H) and IC_{50} is the value of I at which $Y = 0.5$. The dissociation constant of the glycopyrrolate-receptor complex K_i is calculated by dividing IC_{50} by $(1 + Q/K_d)$ where Q is the concentration of the radioligand. Table 2 shows the K_i and n_H values for glycopyrrolate competing vs [3 H] PZ and [3 H] AF-DX 384. Results indicated that glycopyrrolate can displace [3 H] AF-DX 384 ($K_i = 0.03$ nM) more readily than [3 H] PZ ($K_i = 0.60$ nM) from their receptors. The n_H value for glycopyrrolate vs [3 H] PZ was larger than unity at $P < 0.05$ level; suggesting positive cooperativity at the radioligand-receptor complexes. In contrast, the n_H value vs [3 H] AF-DX 384 was not different from unity, indicating a simple competitive interaction at the M_2 site.

DISCUSSION

Glycopyrrolate appears to be a weak inhibitor ($EC_{50} = 0.24$ mM) of the atrial force of contraction. The suppression seems to differ from that induced by the K^+ channel openers, cromakalim and pinacidil (Lau, 1992) which could not completely abolish atrial contraction. This negative inotropic effect of glycopyrrolate does not arise from the blockade of the muscarinic ACh receptor since at concentrations far lower than its EC_{50} , all receptors would be blocked (see Table 2). Rather, the inhibition of atrial contrac-

tion resembles that of Ca^{2+} channel blockers such as diltiazem and nifedipine; both of which can totally relax atrial muscle (Fleckenstein *et al.*, 1984). The antagonism by the Ca^{2+} agonist Bay K 8644 on the negative inotropic effect of glycopyrrolate appears to support such a contention (Fig. 1). Although classified as an antimuscarinic (Goodman and Gilman, 1966), it is plausible that glycopyrrolate could have a different pharmacological spectrum at different concentrations. At concentrations in the millimolar range, it may block Ca^{2+} channels; decrease the slow inward current carried by Ca^{2+} plus Na^+ (Reuter, 1979) and subsequently reduce the force of atrial contraction. Radioligand binding studies with Ca^{2+} channel blockers such as [3 H] nitrendipine (Freeman *et al.*, 1985) may assist in providing some clues. The positive inotropic effect of glycopyrrolate at the lower concentrations was inexplicable as the effect was relatively insignificant and was independent of the concentration of glycopyrrolate.

The depressant effects of acetylcholine and carbachol are both antagonized by glycopyrrolate. Analysis by Schild plot showed linear relationships ($r \leq 0.95$) and the intercept on the abscissa (i.e. dose ratio = 2) was 6.9 nM glycopyrrolate against carbachol and 4.1 nM against acetylcholine. These two apparent dissociation constants are comparable to each other, suggesting that glycopyrrolate and the two muscarinic agonists could interact at a common site. The site is most likely the atrial muscarinic acetylcholine receptor as it is known that these two agonists induce the contractile effects through activating the muscarinic receptors and the associated K^+ channels in the atrium (Freeman, 1974). The slopes of the Schild plot against both agonists were significantly ($P < 0.05$) larger than unity, which are not consistent with simple competitive inhibition. These 'greater than unity' Schild-regression slopes

Table 2. Inhibitory effects of glycopyrrolate on the bindings of [3 H] PZ and [3 H] AF-DX 384 to the muscarinic receptors in guinea-pig brain membranes. Values of K_i and n_H are means $\pm$ SEM. Each data point is calculated from 3-7 experiments.

Radioligand	K_i (nM)	n_H
[3 H] PZ	0.60 $\pm$ 0.22	1.6 $\pm$ 0.11*
[3 H] AF-DX 384	0.03 $\pm$ 0.01	1.3 $\pm$ 0.25

K_i = Binding affinity constant; n_H = Hill coefficient.
* $P < 0.05$.

K_d of [3 H] PZ and [3 H] AF-DX 384 used for K_i calculations were 0.29 and 0.12 nM respectively. The K_d s were obtained from saturation isotherm experiments and estimated by MxN-Fit program (Burginier, 1988).

could be an indication that the drug-receptor interaction is rate limiting, or there is a saturable removal mechanism in the tissue for the antagonist (Kenakin, 1987). Furthermore, it is plausible that glycopyrrolate blocks the muscarinic receptors and also interacts with the associated allosteric site.

Antagonist combination experiments were carried out with QNB, a muscarinic competitive antagonist (Birdsall and Hulme, 1976); gallamine, an allosteric antimuscarinic agent at the cardiac muscarinic receptors (Mitchelson, 1988) and methoctramine, a cardiac M_2 muscarinic antagonist (Melchiorre, 1989). A combination of glycopyrrolate and QNB resulted in dose ratio greater than the sum of the dose ratios produced by the two antagonists when tested alone (Table 1). These results did not seem to be completely consistent with the two antagonists interacting at two different but independent sites or two different sites that subserve the same effector (Paton and Rand, 1965; Clark and Mitchelson, 1976). In contrast, the mutual competition between glycopyrrolate and methoctramine or gallamine gave dose ratios equal to the sum of the dose ratios of the two antagonists individually determined. This strongly suggested that glycopyrrolate competes with methoctramine at the M_2 -muscarinic receptors and with gallamine at the allosteric site. It is possible that glycopyrrolate has different binding sites in cardiac tissues. It may have a high affinity binding to the M_2 subtype, intermediate affinity at the allosteric site and low affinity at the Ca^{2+} channel. Since the cardiac muscarinic receptors are dominated by the M_2 subtype (Vickory *et al.*, 1986), it is conceivable that the M_2 receptors are the primary site for interaction particularly when the competing antagonist is M_2 selective. The radioligand binding studies tend to support this argument. Glycopyrrolate effectively displaced the binding of the M_2 selective [3H] AF-DX 384 (Entzeroth and Mayer, 1990) from the muscarinic M_2 receptors which was approx. 20 times more potent than the displacement of [3H] PZ from the M_1 sites (see Table 2).

In conclusion, it was demonstrated that glycopyrrolate antagonized the depressant responses in guinea-pig atrium induced by two muscarinic agonists. This effect would have been mediated through the blockade of the atrial muscarinic receptors. Radioligand binding studies showed that glycopyrrolate is a potent inhibitor at both the M_1 and M_2 receptors with a preference for the M_2 subtype. This makes glycopyrrolate a potential ligand for muscarinic receptor classification. The mutual competition experiments confirmed that glycopyrrolate interacts competitively with M_2 selective antagonists at the M_2 sites which are the predominant subtype in the atrial tissue. In addition, glycopyrrolate appears to have an allosteric effect and blocks Ca^{2+} channels in the atrium, although the concentration required for Ca^{2+} channel blockade is probably too high to build up under normal physiological conditions. The therapeutic mechanisms of glycopyrrolate treatment of organophosphate poisoning are mainly associated with its potent anti-muscarinic properties. Whether these effects are pertinent to the peripheral system only or have some implications in the CNS is still controversial as there are reports indicating central

anticholinergic symptoms following glycopyrrolate administration (Grum and Osborne, 1991).

Acknowledgements—The authors wish to thank A. H. Robbins Ltd, Sydney for a gift of glycopyrrolate. We are particularly grateful to Dr Shirley Freeman for reviewing this article.

REFERENCES

- Arunlakshana O. and Schild H. O. (1959) Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* 14, 48–58.
- Birdsall N. J. M. and Hulme E. C. (1976) Biochemical studies on muscarinic acetylcholine receptors. *J. Neurochem.* 27, 7–16.
- Burgisser E. (1988) MxN-FIT, an IBM-PC programme for the analysis of complex binding data according to the law of mass action, pp. 1–111. Bureco AG, Kaiserstrasse 5a CH-4310, Rheinfelden, Switzerland.
- Clark A. L. and Mitchelson F. (1976) The inhibitory effect of gallamine on muscarinic receptors. *Br. J. Pharmacol.* 58, 323–331.
- Downie N. M. and Heath R. W. (1970) *Basic Statistical Methods*, pp. 178–185. Harper International Edition, Harper & Row, New York.
- Entzeroth M. and Mayer N. (1990) Labelling of rat heart muscarinic receptors using the new M_2 selective antagonist [3H] AF-DX 384. *Biochem. Pharmacol.* 40, 1674–1676.
- Fleckenstein A., Van Breemen C., Grop R. and Hoffmeister F. (1984) *Cardiovascular Effects of Dihydropyridine-Type Calcium Antagonists and Agonists*, pp. 3–31. Springer, Berlin.
- Freeman S. E. (1974) Cholinergic mechanisms in heart: interactions with 4-aminopyridine. *J. Pharmacol. exp. Ther.* 210, 7–14.
- Freeman S. E. and Turner R. J. (1974) Phase-plane trajectories of atrial cell action potentials: effects of temperature reduction. *Cardiovasc. Res.* 8, 451–459.
- Freeman S. E., Dawson R. M., Culvenor A. J. and Keeghan A. M. (1985) Interactions of amantadine with the cardiac muscarinic receptor. *J. Mol. Cell. Cardiol.* 17, 9–21.
- Freeman S. E., Dawson R. M. and Keeghan A. M. (1986) Modification of the effects of muscarinic agonists by reversible and irreversible anticholinesterase compounds in the guinea-pig atrium. *J. Mol. Cell. Cardiol.* 18, 231–239.
- Goodman L. S. and Gilman A. (1966) *The Pharmacological Basis of Therapeutics*, 3rd edn, p. 534. Macmillan, New York.
- Grum D. F. and Osborne L. R. (1991) Central anticholinergic syndrome following glycopyrrolate. *Anesthetics* 74, 191–193.
- Kenakin T. P. (1987) *Pharmacologic Analysis of Drug Receptor Interaction*, pp. 205–244. Raven Press, New York.
- Lau W. M. (1992) The effects of potassium channel blockers on the negative inotropic responses induced by cromakalim and pinacidil in guinea-pig atrium. *Pharmacology* 45, 9–16.
- Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Melchiorre C., Quaglia W., Picchio M. T., Giardina D., Brasili L. and Angeli P. (1989) Structure-activity relationships among methoctramine-related polymethylene tetramines. Chain-length and substituent effects on M_2 muscarinic receptor blocking activity. *J. Med. Chem.* 32, 79–84.
- Mitchelson F. (1988) Muscarinic receptor differentiation. *Pharmacol. Ther.* 37, 357–423.
- Muravchick S., Owens W. D. and Felts J. A. (1979) Glycopyrrolate and cardiac dysrhythmias in geriatric

- patients after reversal of neuromuscular blockade. *Can. Anaesth. Soc. J.* 26, 22-25.
- Paton W. D. M. and Rang H. P. (1965) The uptake of atropine and related drugs by intestinal smooth muscle of the guinea-pig in relation to acetylcholine receptors. *Proc. R. Soc. Lond. Ser. B.* 163, 1-44.
- Proakis A. G. and Harris G. B. (1979) Comparative penetration of glycopyrrolate and atropine across the blood-brain and placental barriers in anaesthetised dogs. *Anesthetics* 48, 338-344.
- Reuter H. (1979) Properties of two inward membrane currents in the heart. *A. Rev. Physiol.* 41, 413-424.
- Richards D. L. S., Clutton R. E. and Boyd C. (1989) Electrocardiographic findings following intravenous glycopyrrolate to sedated dogs: a comparison with atropine. *J. Ass. Vet. Anaesth.* 16, 46-50.
- Tracey J. A. and Gallagher H. (1990) Use of glycopyrrolate and atropine in acute organophosphorus poisoning. *Human exp. Toxicol.* 9, 99-100.
- Vickroy T. W., Roeske W. R. and Yamamura H. I. (1986) [³H] Pirenzepine and (-)-[³H] quinuclidinyl benzilate binding to rat cerebral cortical and cardiac muscarinic cholinergic sites. II. Characterisation and regulation of antagonist binding to putative muscarinic subtypes. *J. Pharmac. exp. Ther.* 237, 419-427.
- Wali F. A., Clark C. H., Altinel A. and Jones C. J. (1987) Glycopyrrolate intensifies neuromuscular blockade produced by atracurium in the rat diaphragm preparation. *Pharmac. Res. Commun.* 19, 827-835.
- Wali F. A., Suer A. H., McAteer E., Dark C. H. and Jones C. J. (1988) Effects of atropine and glycopyrrolate on neuromuscular transmission in the rat phrenic nerve-diaphragm preparation. *Gen. Pharmac.* 19, 285-290.
- Watson M., Roeske W. R., Vickroy T. W., Smith T. L., Akiyama K., Gulya K., Duckles S. P., Serra M., Adem A., Nordberg A., Gehlert D. R., Wamsley J. K. and Yamamura H. I. (1986) Biochemical and functional basis of putative muscarinic receptor subtypes and its implications. *Trends Pharmac. Sci. Suppl. Subtypes of Muscarinic Receptors* 46-55.

DTIC QUALITY INSPECTED 5

Accession For	
NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	21