*Br. J. Pharmac.* (1985), **86**, 609–617

# Pharmacological analysis of the muscarinic receptors involved when McN-A 343 stimulates acid secretion in the mouse isolated stomach

J.W. Black & N.P. Shankley

The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5 8RX

**1** In view of the recent $M_1$ and $M_2$ subclassification of muscarinic receptors and the suggestion of separate populations of muscarinic receptors on oxyntic and histamine cells in the gastric mucosa, we have analysed the effects of McN-A 343, classified as an $M_1$-selective agonist, on gastric acid secretion by the mouse, isolated, lumen-perfused stomach assay.

**2** Acid secretion stimulated by McN-A 343 was not inhibited by tetrodotoxin pretreatment, although it was competitively antagonized by atropine ($pK_B$ 7.90), suggesting a muscarinic site of action between postganglionic neurones and the final secretory event.

**3** Acid secretion stimulated by McN-A 343 was more sensitive than 5-methylfurmethide-stimulated secretion to $H_2$-receptor blockade: the profile of inhibition was consistent with expectations for a model of indirect agonism, suggesting that McN-A 343 preferentially stimulated the release of endogenous histamine from mucosal histamine cells.

**4** In view of this selective action the McN-A 343-pirenzepine interaction was studied, the latter being classified as an $M_1$-selective antagonist. Results were consistent with expectations for a competitive interaction but the $pK_B$ (6.69) was not significantly different from the value obtained at the oxyntic cell, using 5-methylfurmethide as agonist in the presence of $H_2$-receptor blockade, in a previous study.

**5** We suggest that there is no need to postulate differences in oxyntic and histamine cell muscarinic receptors to account for the selective stimulant activity of McN-A 343 observed in this study and the relatively selective inhibition of gastric acid secretion by pirenzepine *in vivo*. McN-A 343 selectivity may be accounted for by a higher muscarinic receptor density on the histamine cell and pirenzepine selectivity by a smaller degree of loss into the gastric secretion compared to atropine.

## Introduction

Angus & Black (1982) found that acid secretion by the isolated, lumen-perfused, stomach preparation of the mouse could be evoked by electrical field stimulation. This secretion appeared to be mediated by vagal nerve endings because treatment with either tetrodotoxin or atropine could abolish the response. Surprisingly, this response could also be abolished by metiamide, using concentrations at which selective histamine $H_2$-receptor blockade only was expected. Shankley (unpublished results) has recently confirmed this discovery using tiotidine. These observations have strengthened the idea that acetylcholine (ACh) released from vagal nerve endings activates muscarinic receptors on histamine cells: the released histamine is then imagined to act on $H_2$-receptors on the oxyntic cells to stimulate acid secretion. The phenomenon has still to be confirmed in other species. Nevertheless, the hypothesis is essentially a restatement of the view originally proposed by MacIntosh (1938).

What made the inhibition of field stimulation by metiamide surprising was that metiamide had previously been shown to be unable to inhibit the secretory effects of bethanechol in the mouse stomach preparation (Angus & Black, 1982). The refractoriness to metiamide of oxyntic cell secretion evoked by cholinoceptor (muscarinic) activation was well known. The existence of muscarinic receptors on oxyntic cells had been demonstrated both by functional studies (Soll, 1980; Ecknauer *et al.*, 1981) and by [$^3$H]-quinuclidinyl benzilate (QNB) binding measurements (Ecknauer *et al.*, 1980). Unlike histamine receptors on oxyntic cells, which had been shown to be coupled to adenylate cyclase (Scholes *et al.*, 1976), there was evidence that the muscarinic

receptors operated a different transducer mechanism (see Soll, 1981). Finally, the inability of $H_2$-receptor blockade to suppress cholinergic secretion had been shown unequivocally in studies with dog isolated oxyntic cells (Soll, 1980), rabbit isolated gastric glands (Berglindh, 1977) and cat isolated gastric muscosal sheets (Tepperman *et al.*, 1975). On the other hand, carbachol-stimulated acid secretion in the mouse stomach was found to show some sensitivity to $H_2$-receptor blockade: metiamide achieved a dose-ratio of nearly 1 log unit (Angus & Black, 1982). However, as this effect of metiamide was blocked by hexamethonium, it was argued that carbachol stimulated ganglionic nicotinic receptors as well as oxyntic cell muscarinic receptors: ganglionic stimulation was imagined to release histamine in the same way as had been found with electrical field stimulation.

The results with carbachol reinforced the problem raised by field stimulation. Both interpretations postulated the existence of muscarinic receptors on the histamine cells as well as on the oxyntic cells. Therefore, how could neurally-released (endogenous) ACh activate mainly histamine cell receptors and exogenous bethanechol apparently activate predominantly the oxyntic cell receptors? The solution proposed by Angus & Black (1982) to the first part of the problem was essentially structural: the selectivity of endogenous ACh for histamine cell receptors was to be due, anatomically, to the propinquity of nerve terminals to histamine cells rather than to oxyntic cells. In addition, there was some evidence from binding studies that oxyntic cells were sparsely populated with muscarinic receptors, hinting at the possibility that the histamine cells were more richly endowed with them.

This solution begged a number of points, not least the failure to explain why the effects of exogenous choline esters failed to exhibit a metiamide-sensitive component. This aspect of the problem has been solved by a recent study (Black & Shankley, 1985b) using an improved bioassay to analyse the muscarinic receptors coupled to acid secretion. The concentration-effect curve to 5-methylfurmethide (5mef), a highly potent and selective muscarinic agonist, could be displaced to the right by tiotidine, a potent and selective $H_2$-receptor antagonist.

The important point, from both a theoretical and technical point of view, is that the maximum dose-ratio achieved by tiotidine, namely 2.1, simply could not have been detected by the earlier experiments based on a 2 + 2 assay design – the variances were too great. However, although this dose-ratio is small, intuitively (see Discussion) this is the expected result where the system is expressing the consequences of small differences in receptor density between histamine and oxyntic cells. The control concentration-effect curve is mainly due to histamine release. After $H_2$-receptor blockade, doubling the concentration of the muscarinic agonist is enough to activate the oxyntic cell receptors directly.

These attempts to account for the effects of muscarinic receptor agonists have assumed that muscarinic receptors on different types of cells are homogeneous. However, a strong case has been made out recently (Giachetti *et al.*, 1982; Hammer & Giachetti, 1984) for the view that muscarinic receptors are not an entity. The extent of the proposed heterogeneity has still to be clarified but there is wide agreement that at least two receptor types, $M_1$ and $M_2$, can be identified by both pharmacological and radioligand binding studies.

In this view, pirenzepine, a tricyclic muscarinic receptor antagonist, has been classified as selective for $M_1$-receptors. Corresponding to this, McN-A 343, a muscarinic agonist, long recognized as a selective activator of sympathetic ganglion cells, has now been classified as a selective $M_1$-receptor agonist (Giachetti *et al.*, 1982).

In practice, pirenzepine has been assigned the property of being able to inhibit acid secretion with fewer effects on vision and salivation than other muscarinic receptor antagonists thus making it useful in the treatment of peptic ulcer disease (Londong & Londong, 1982). On the other hand, Odori & Magee (1969) found that McN-A 343 greatly augmented pentagastrin but not histamine or methacholine-stimulated gastric acid secretion. In the light of the new hypothesis about the heterogeneity of muscarinic receptors, this older observation suggests that the histamine cell receptors might be $M_1$ and different from those on oxyntic cells.

In an attempt to classify the nature of muscarinic receptors involved in the induction of acid secretion, this paper analyses the effects of McN-A 343 on gastric acid secretion by the isolated stomach preparation of the mouse.

## Methods

### *Acid secretion*

Acid secretion was measured in the isolated, lumen-perfused, stomach preparation of the mouse as described previously (Black & Shankley, 1985a). Briefly, stomach preparations from fasting mice were established with the pH electrode system arranged to provide a 12 cm$H_2O$ intragastric pressure. Six preparations were used simultaneously and after an initial 60 min stabilization period those not producing a stable basal acid secretion (approximately 5%) were rejected. All drugs were added directly to the organ bath (serosal side) and, following a further 60 min equilibrium period in the absence or presence of antagonist, a single cumulative concentration-effect curve was obtained.

*Experimental design*

Antagonist treatments were allocated in a block design such that, as far as possible, all organ baths received each treatment during the course of an experiment.

*Analysis*

Acid secretion responses resulting from the addition of agonists were measured as the change in pH (ΔpH) of the lumen perfusate measured from the baseline immediately before starting the agonist concentration-effect curve. The curve data from individual preparations were fitted to a logistic function as described previously (Black & Shankley, 1985a). For display purposes the individual computed estimates of the parameters governing the function for each treatment group were expressed as means and a single curve generated and superimposed upon the experimental data.

Agonist-antagonist interactions were analysed as described previously (Black *et al.*, 1985a).

*Drugs*

Drugs were freshly prepared in distilled water. The total volume added to the 40 ml organ bath did not exceed 400 μl. Drugs and their sources were as follows: N-methylatropine (Sigma), atropine (Sigma), 5-methylfurmethide (5mef; Wellcome Research Laboratories) and tetrodotoxin (Sigma). McN-A 343, tiotidine and pirenzepine were generous gifts from McNeil Laboratories USA, Imperial Chemical Industries Ltd and A.B. Hässle Ltd, respectively.

## Results

*McN-A 343 concentration-effect curves*

Individual secretory responses to McN-A 343 reached a sustained plateau after approximately 15 min and a fully-defined cumulative concentration-effect curve (Figure 1) could be obtained in a single stomach preparation. Pretreatment with tetrodotoxin (TTX) 0.1 μM for 60 min has been found to abolish totally the responses to electrical field stimulation in the mouse stomach (Angus & Black, 1982). Therefore, because of the original classification of McN-A 343 as a ganglionic stimulant (Roszkowski, 1961), we expected that TTX would also block gastric acid secretion stimulated by McN-A 343 on the assumption that its effects were due to ganglionic stimulation of the parasympathetic innervation of the gastric mucosa. However, TTX (0.1 μM for 60 min) did not affect McN-A 343 concentration-effect curves (Figure 2). Apparently McN-A 343 stimulates gastric acid secretion independently of propagated neuronal action potentials and, therefore, ganglionic activity.

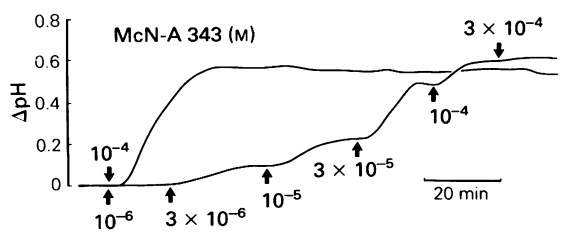



**Figure 1** Experimental record of a single response and cumulative concentration-effect curve obtained with McN-A 343 on the mouse stomach assay. ΔpH (ordinate scale) refers to the change in pH of the lumen perfusate. Doses of McN-A 343 were added when responses reached a plateau to give the organ bath concentration indicated.

*The effect of atropine on McN-A 343 concentration-effect curves*

To determine whether McN-A 343 was acting on muscarinic receptors to stimulate acid secretion the interaction between McN-A 343 and atropine was examined. Our previous analysis of muscarinic receptors coupled to oxyntic cell secretion (Black & Shankley, 1985b) made use of the potency and selectivity of 5mef to explore a wide range of muscarinic antagonist concentrations (dose-ratios up to 3,000). However, due to the relatively low potency of McN-A 343 ($[A_{50}] = 6$ μM) and depressant effects on acid secretion at concentrations above 3 mM, the range of antagonist

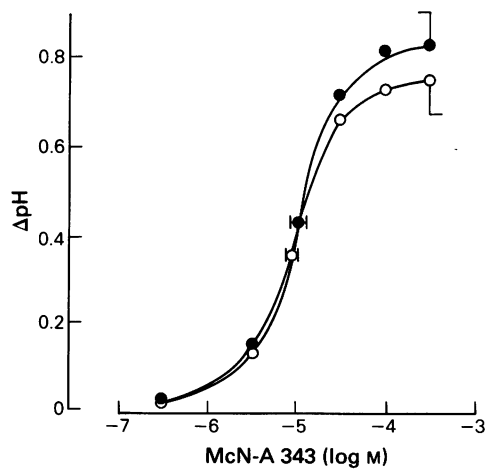



**Figure 2** McN-A 343 concentration-effect curves obtained in the absence (●) and presence (○) of tetrodotoxin (0.1 μM for 60 min) on the mouse stomach assay. The curves drawn through the mean experimental data points ($n = 6$) were obtained for a logistic fitting procedure. Error bars show standard errors.

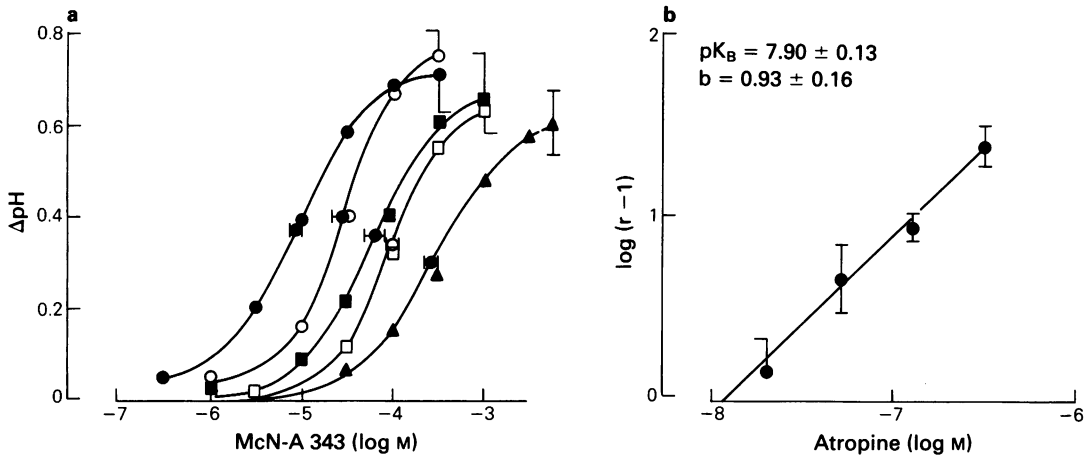



**Figure 3** (a) McN-A 343 concentration-effect curves obtained on the mouse stomach assay in the absence (●) and presence of atropine (○) $2 \times 10^{-8}$ (■) $5 \times 10^{-8}$ (□) $1.25 \times 10^{-7}$ and (▲) $3 \times 10^{-7}$M. The curves drawn through the mean experimental data points ($n = 5/6$) were obtained from a logistic curve-fitting procedure. Error bars show standard errors. (b) Schild plot for McN-A 343/atropine on the mouse stomach. Dose-ratios (r) were calculated from the mean [$A_{50}$] values estimated for the McN-A 343 concentration-effect curves. Estimates of slope parameter (b ± s.e.) and $pK_B$ (± s.e.) were obtained by model fitting.

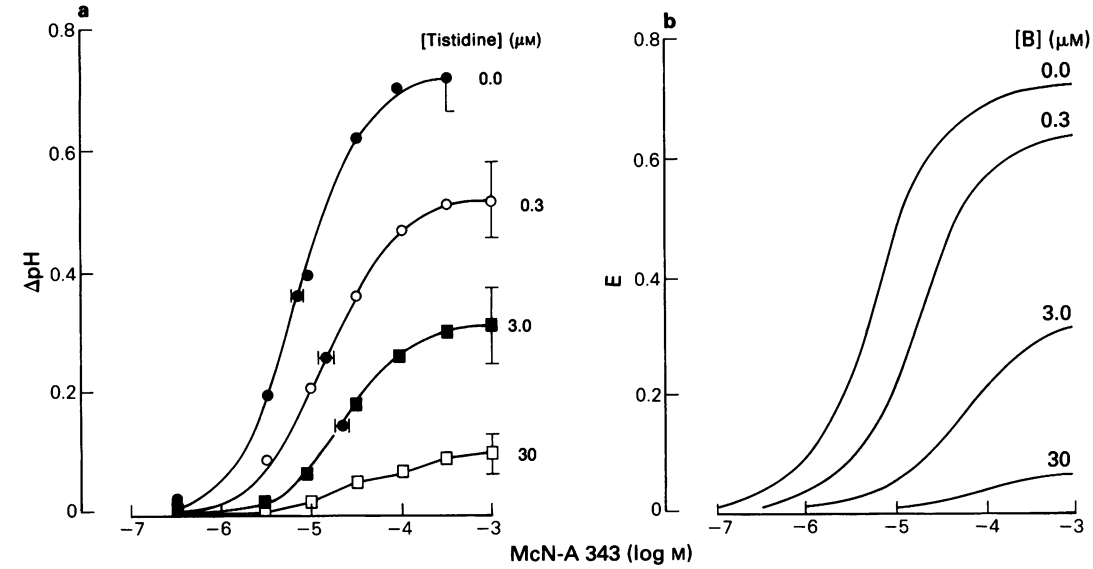



**Figure 4** (a) McN-A 343 concentration-effect curves obtained on the mouse stomach assay in the absence (●) and presence of tiotidine (○) $3 \times 10^{-7}$. (■) $3 \times 10^{-6}$ and (□) $3 \times 10^{-5}$M. The curves drawn through the mean experimental data points ($n = 5/6$) were obtained from a logistic curve-fitting procedure. Due to almost maximal inhibition of some of the McN-A 343 curves in the presence of $3 \times 10^{-5}$M tiotidine, it was not possible to fit individual logistic curves to these data. Error bars show standard errors. (b) Simulation of the McN-A 343/tiotidine interaction. The concentration-effect curve data shown in (a) were analysed using the theoretical model describing the competitive antagonism of the mediator released by an indirectly acting agonist (Black *et al.*, 1985a). Model fitting gave the following parameter estimates: n = 1.14, $K_G = 1.06 \times 10^{-4}$M, $E_M = 0.74$ and τ values of 22.2 (control), 5.9 ($3 \times 10^{-7}$M tiotidine), 0.78 ($3 \times 10^{-6}$M tiotidine) and 0.08 ($3 \times 10^{-5}$M tiotidine). These parameter values were used to generate the concentration-effect curves shown.

concentrations studied was restricted such that dose-ratios of only 30 could be determined with corresponding full definition of concentration-effect curves. Nevertheless, atropine produced a significant concentration-dependent parallel displacement of the McN-A 343 concentration-effect curves with no significant change in the maximal asymptote (Figure 3). Competitive analysis (Black *et al.*, 1985a) showed that the slope of the Schild regression was not significantly different from unity. The estimate of $pK_B$ (7.90 ± 0.13) was not significantly different from that obtained with atropine and 5mef ($pK_B$ = 7.78 ± 0.09) in the analysis of muscarinic receptors coupled to oxyntic cell secretion (Black & Shankley, 1985b). Therefore, accepting the hypothesis that the difference in $pK_B$ estimates for muscarinic receptor antagonists between the mouse stomach and other visceral muscle assays is due to the steady-state loss of antagonist from the receptor compartment to the acid secretion in the stomach preparation (Angus & Black, 1979; Black & Shankley, 1985b), it would appear to be reasonable to conclude that McN-A 343 and 5mef are acting on the same class of muscarinic receptors to stimulate gastric acid secretion in the mouse stomach.

*The effect of $H_2$-receptor blockade on McN-A 343 concentration-effect curves*

The $H_2$-receptor antagonist, tiotidine, significantly displaced the McN-A 343 concentration-effect curve with concomitant depression of the maximal asymptote (Figure 4a). This effect of $H_2$-receptor blockade is different from that produced on the concentration-effect curves of 5mef, bethanechol or carbachol (Black & Shankley, 1985b; Angus & Black, 1982) where the same maximal stimulation of acid secretion in the absence and presence of $H_2$-blockade could be obtained. On the other hand, this pattern of inhibition is similar to that found with $H_2$-receptor blockade of acid secretion stimulated by either pentagastrin (Black *et al.*, 1985b) or vagal activation (Angus & Black, 1982). Therefore, this finding suggests that McN-A 343 stimulates acid secretion in the mouse stomach predominately by releasing histamine.

Accordingly, the McN-A 343-tiotidine experimental data have been analysed using the theoretical model developed to describe the competitive antagonism of the mediator released by an indirectly-acting agonist (Black *et al.*, 1985b). McN-A 343 is assumed to release endogenous histamine which acts on $H_2$-receptors to stimulate oxyntic cell secretion in the same way as exogenously administered histamine. Tiotidine is assumed to compete with histamine for $H_2$-receptors. The parameters obtained from direct fitting of the experimental data to the model were used to simulate the concentration-effect curves in Figure 4b. Apparently the theoretical model provides a reasonable description of the experimental data obtained.

These results suggest that McN-A 343 differs from other muscarinic agonists administered to the mouse stomach preparation in that its effects are largely mediated by histamine release and that it does not possess an equivalent ability to stimulate directly the muscarinic receptors coupled to oxyntic cell secretion.

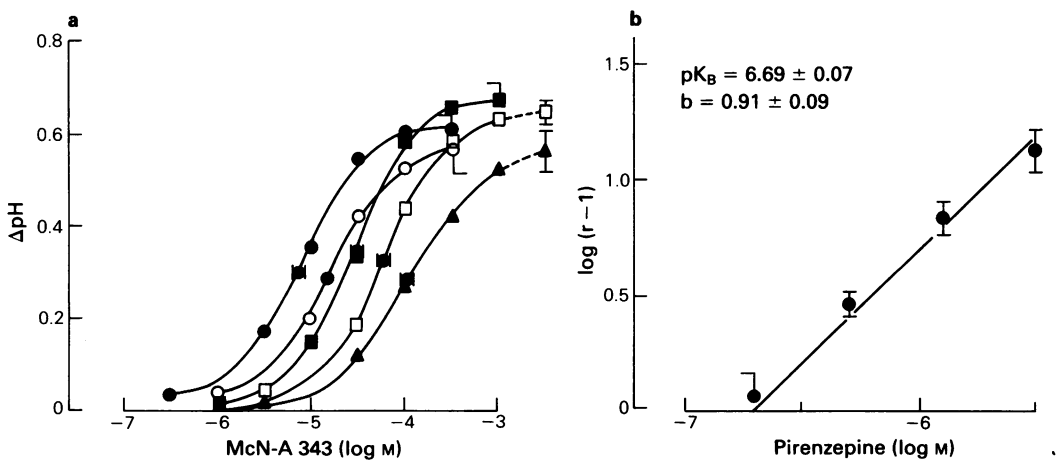



**Figure 5** (a) McN-A 343 concentration-effect curves obtained on the mouse stomach assay in the absence (●) and presence of pirenzepine (○) $2 \times 10^{-7}$, (■) $5 \times 10^{-7}$, (□) $1.25 \times 10^{-6}$ and (▲) $3 \times 10^{-6}$ M. The curves drawn through the mean experimental data points ($n$ = 5/6) were obtained from a logistic curve fitting procedure. Error bars show standard errors. (b) Schild plot for McN-A 343/pirenzepine assay on the mouse stomach. Dose-ratios (r) were calculated from the mean [$A_{50}$] values estimated for the McN-A 343 concentration-effect curves. Estimates of slope parameter (b ± s.e.) and $pK_B$ (± s.e.) were obtained by model fitting.

A reasonable conclusion is that McN-A 343 is a selective ligand for investigating the muscarinic receptors mediating histamine release in the mouse stomach. This would support the proposal of Giachetti *et al.* (1982) that McN-A 343 is a selective $M_1$-receptor agonist. In that case, pirenzepine should be a selective antagonist of McN-A 343 as these authors also proposed.

*The effect of pirenzepine on McN-A 343 concentration-effect curves*

In a previous paper (Black & Shankley, 1985b) we compared the activity of the muscarinic receptor antagonist, pirenzepine, in the guinea-pig trachea assay and on muscarinic receptors coupled to oxyntic cell secretion in the mouse stomach. Pirenzepine did not appear to demonstrate any selectivity for the oxyntic cell muscarinic receptor. The *in vivo* gastric acid selectivity of pirenzepine may, of course, be due to blockade of muscarinic receptors remote from the oxyntic cell but still involved in the control of gastric acid secretion. The apparent selective action of McN-A 343 on the histamine-cell muscarinic receptors in the mouse stomach revealed in this study, provided an opportunity to determine the affinity of pirenzepine at these muscarinic receptors.

Pirenzepine produced a significant concentration-dependent parallel displacement of the McN-A 343 concentration-effect curve without significant change in the maximal asymptote (Figure 5). Competitive analysis indicated a Schild regression not significantly different from unity. The estimate of $pK_B$ ($6.69 \pm 0.07$) was not significantly different from that obtained from the interaction with 5mef, in the presence of $H_2$-receptors blockade, on the oxyntic cell muscarinic receptor ($pK_B = 6.67 \pm 0.09$; Black & Shankley, 1985b). Therefore, the muscarinic receptors on the histamine and oxyntic cells cannot be differentiated by the expressed binding affinities of either atropine or pirenzepine.

## Discussion

The muscarinic agonist, McN-A 343, was originally described as a selective stimulant of receptors on sympathetic ganglion cells (Roszkowski, 1961). Since then, unambiguous evidence for a similar action on parasympathetic ganglion cells has not been achieved. The finding in this paper that the stimulant effects of McN-A 343 on acid secretion are not annulled by tetrodotoxin might be seen as evidence that parasympathetic ganglion cells are not involved. However, the sensitivity of McN-A 343 to blockade by atropine predicates that muscarinic receptor activation is nevertheless the basis for the agonism. Therefore, if the current ideas are substantially correct, these muscarinic receptors must lie somewhere between the parasympathetic postganglionic neurones and the final secretory event. These muscarinic receptors would then be in series with muscarinic receptors on the parasympathetic ganglion cells, should they exist. If, now, tetrodotoxin eliminated the consequences of activation of these ganglion cell receptors, McN-A 343 would then activate solely the cells with the second population of receptors. There would be no loss of sensitivity provided the cellular receptor densities in the two sites were roughly equal. Therefore, failure to displace McN-A 343 concentration-effect curves with TTX is not evidence against parasympathetic ganglion involvement. Nevertheless, the effects of atropine point to at least an additional locus of muscarinic receptors which McN-A 343 can activate on non-neural cells.

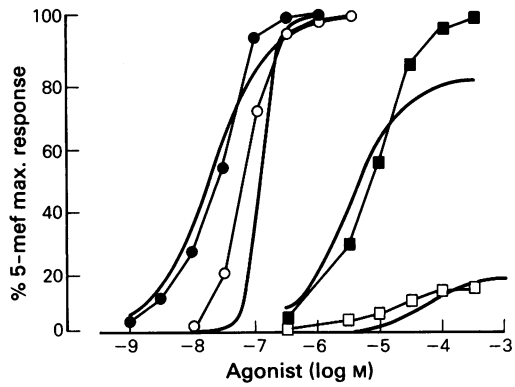
The existence of muscarinic receptors on oxyntic cells is well established and the effects of their activation cannot be inhibited by $H_2$-receptor antagonists (see Introduction). The concentration-effect curves to bethanechol or 5mef on the isolated stomach preparation of the mouse can be displaced by no more than a factor of 2 by tiotidine. Therefore the finding that tiotidine displaces the McN-A 343 concentration-effect curves with a combination of right shift and reduction of the maximum argues against McN-A 343 having its primary agonist action at oxyntic cell muscarinic receptors. Moreover, as tiotidine displaces pentagastrin curves in the same way, this argues for an action of McN-A 343 on the histamine cells (Black *et al.*, 1985b). At the same time, this evidence requires that McN-A 343 has, compared to bethanechol and 5mef, a differential sensitivity to the receptors on the histamine and oxyntic cells. Giachetti *et al.* (1982) have proposed that McN-A 343 is a selective muscarinic $M_1$-receptor agonist and this classification could account for the selectivity if the histamine and oxyntic cells expressed $M_1$- and $M_2$-receptors respectively on their membranes. This possibility can be tested by measuring the parameters of the interaction between McN-A 343 and pirenzepine because pirenzepine is now classified as a selective $M_1$-receptor antagonist.

The effects of pirenzepine on McN-A 343 concentration-effect curves could not be distinguished from simple competitive antagonism. A $pK_B = 6.69 \pm 0.07$ was calculated for this action of pirenzepine postulated to be at muscarinic receptors on histamine cells. This value is not significantly different from the value ($pK_B = 6.67 \pm 0.09$) calculated for the interaction between pirenzepine and 5mef in the presence of tiotidine. Under these conditions, 5mef is imagined to activate oxyntic cell muscarinic receptors (Black & Shankley, 1985b). Similarly, no significant difference was found between the $pK_B$ values for atropine with

McN-A 343 and 5mef plus tiotidine measured on the isolated stomach preparation of the mouse. Therefore, judged by the estimated dissociation constants for pirenzepine and atropine, the muscarinic receptors on histamine and oxyntic cells belong to the same class.

If the muscarinic receptors on histamine cells and oxyntic cells belong to the same class, then McN-A 343 should have identical affinity for the receptors on each type of cell. Therefore, the selectivity which McN-A 343 apparently displays for the histamine cells would have to be due to a higher expressed efficacy at these cells. A phenomenon like this has been studied by Kanakin & Beek (1980). They showed that prenalterol (a β-adrenoceptor agonist), expressed widely varying degrees of efficacy in six different tissues. They attributed the variations between tissues to differences in the stimulus-effect coupling parameter, β, in the traditional receptor-stimulus model of agonism. However, an alternative approach to that problem was suggested in a recently developed general model of agonism (Black & Leff, 1983). In this model the efficacy of the agonism expressed in an effector system is attributed to the ratio $[R_o]/K_E$, defined as the transducer ratio (τ), where $[R_o]$ is the total receptor density and $K_E$ is the location parameter for the relation between the concentration of occupied receptors [AR] and the effect (E). In the simple receptor-transducer version of that model, $K_E = K_{AR}$ and 'the only parameter available to account for intertissue variations in agonism is $[R_o]$'. In the complex receptor-transducer version, a stimulus-effect coupling parameter, β, is also needed. Nevertheless, when the data of Kenakin & Beek were fitted to the complex model, variation in $[R_o]$ alone was sufficient to account for the differences in the expressed efficacy of prenalterol. The 'main reason for preferring $[R_o]$ to β as the source of the (intertissue) variation is, in terms of model fitting, because it offers a simpler explanation for the experimental data'. By analogy, the theoretical effect of assigning a relatively higher total muscarinic receptor density to the histamine cells has been investigated.

Although the problem of finding a normalising principle to allow a valid comparison of receptor densities, using the radioligand binding technique, has still to be solved, nevertheless Ecknauer *et al.* (1980) apparently found a very low density of muscarinic binding sites on isolated oxyntic cells compared to values reported by others for visceral muscle (Yamamura & Snyder, 1974) or brain (Wastek & Yamamura, 1978). So that the general model of agonism could be used to test the logical strength of our intuitive judgement that these variations in receptor densities could account for the expressed efficacies of McN-A 343, we have assumed that the histamine cells have a six fold higher density of muscarinic receptors than oxyntic cells. For comparison, we have assumed that 5mef is a full agonist on both tissues, that is that its maximum response is effector-limited rather than receptor-limited in both tissues and there is in effect a receptor reserve. Consequently, the higher receptor density on the histamine cells would only have the effect of increasing the potency of 5mef at these cells relative to oxyntic cells. This would then be consistent with the small dextrad shift of the 5mef concentration-effect curves produced by tiotidine




| | τ value | |
|---|---|---|
| | 5-mef | McN-A 343 |
| Oxyntic cell | 120 | 4 |
| Histamine cell | 20 | 0.67 |

**Figure 6** 5-Methylfurmethide (5mef; circles) and McN-A 343 (squares) experimental concentration-effect curves in the absence (closed symbols) and presence of $H_2$-receptor blockade (open symbols) on the mouse stomach assay and concentration-effect curves simulated (continuous bold line) using the general model of agonist activity (Black & Leff, 1983). Tiotidine is assumed to convert agonist activity due to the effects of released histamine to activity due to direct stimulation of oxyntic cells. The muscarinic receptors on the histamine and oxyntic cells are assumed to be identical and, accordingly, the same $K_A$ values used to simulate each 5mef and McN-A 343 concentration-effect curve. The $K_A$ value for 5mef ($2.4 \times 10^{-6}$M) was that estimated by Leff *et al.* (1985) on the guinea-pig trachea assay and that for McN-A 343 ($1.6 \times 10^{-5}$M) the affinity estimate obtained by Van Rossum (1962) on the rat intestine. Values of τ, defined as $[R_o]/K_E$ in the model were calculated to account for the dextrad shift of the experimental 5mef concentration-effect curve and the shift and depression of the McN-A 343 concentration-effect curve in the presence of tiotidine. A six fold reduction of τ, and, hence, $[R_o]$ was required to provide a reasonable description of the experimental data. The slope parameter, n, introduced in the model to account for non-hyperbolic concentration-effect curves was fixed at 1 for agonist activity due to histamine release and 3.8 for agonist activity directly on the oxyntic cell.

(Black & Shankley, 1985b): by blocking the effects of released histamine, tiotidine is imagined to shift the concentration-effect curve involving histamine cell receptors to a curve involving oxyntic cell receptors. In addition, the 5mef concentration-effect curve was observed to be significantly steeper in the presence of tiotidine. In applying the general model of agonism we have assumed that the function relating oxyntic cell muscarinic receptor occupancy to acid secretion is steep. This is achieved in the model by replacing the hyperbolic transducer function by a logistic function with slope parameter, n, equal in this case to 3.8 (see Black & Leff, 1983). On the other hand, McN-A 343 has been assumed to have an intrinsic efficacy, $K_E$ in the model, sufficiently low that values of [H] large enough to produce maximal responses can only occur with high values of $[R_o]$, the receptor density. If the receptor density on the histamine cells was just sufficient to allow McN-A 343 to express a maximum response, then by our assumption, the lower receptor density on the oxyntic cells would reduce its efficacy there to that of a partial agonist. Therefore, when the McN-A 343 concentration-effect curve is switched from a histamine cell response to an oxyntic cell response by tiotidine, the maximum response will be reduced. A comparison of the model behaviour with the effects of tiotidine on the 5mef and McN-A 343 concentration-effect curves is shown in Figure 6.

A corollary to this proposal, that the selectivity of McN-A 343 could be accounted for by the combination of low intrinsic efficacy plus differences in receptor densities between cell types, is that McN-A 343 should act as a competitive antagonist to 5mef when it is acting directly on the oxyntic cells. Preliminary experiments (data not shown) indicate that McN-A 343 can indeed antagonize the effects of 5mef in the presence of tiotidine to give an estimated $pK_B$ of 4.7. Interestingly enough, Van Rossum (1962) has already shown that McN-A 343 antagonizes the muscarinic agonist, furtrethonium, on the rat intestine ($pA_2 = 4.8$).

Our conclusion is that there is no need to postulate qualitative differences in receptor types to account for the selective effects of McN-A 343 on acid secretion by the mouse stomach. The effects of pirenzepine were important in arriving at this conclusion. Pirenzepine did not distinguish the muscarinic receptors attributable to histamine cells and oxyntic cells from those on tracheal smooth muscle cells (Black & Shankley, 1985b). In fact the only significant difference we have been able to find between the muscarinic antagonist properties of pirenzepine and atropine is that, unlike atropine, the $pK_B$ for pirenzepine on the oxyntic cells is not relatively low. We have attributed this difference to the relative polarity of pirenzepine and the possibility that, like other polar compounds, it is not continuously lost through the oxyntic cells into the gastric juice. The consequence of this is that effective blockade of the muscarinic receptors involved in regulating the secretion of acid might be achieved, *in vivo*, at relatively lower plasma concentrations of pirenzepine compared to those of atropine. This phenomenon alone might be sufficient to account for its tissue selectivity in man.

This work was supported by The Wellcome Foundation Ltd. The authors wish to thank Dr Paul Leff (Department of Pharmacology, The Wellcome Foundation Ltd) for valuable scientific discussion and Mrs H.D. Williams for help in preparing the manuscript.

## References

ANGUS, J.A. & BLACK, J.W. (1979). Analysis of anomalous $pK_B$ values for metiamide and atropine in the isolated stomach of the mouse. *Br. J. Pharmac.*, **67**, 59–65.

ANGUS, J.A. & BLACK, J.W. (1982). The interaction of choline esters, vagal stimulation and $H_2$-receptor blockade on acid secretion *in vitro*. *Eur. J. Pharmac.*, **80**, 217–224.

BERGLINDH, T. (1977). Effects of common inhibitors of gastric acid secretion on secretagogue-induced respiration and aminopyrine accumulation in isolated gastric glands. *Biochem. biophys. Acta*, **464**, 217–233.

BLACK, J.W. & LEFF, P. (1983). Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B*, **220**, 141–162.

BLACK, J.W., LEFF, P. & SHANKLEY, N.P. (1985a). Further analysis of anomalous $pK_B$ values for histamine $H_2$-receptor antagonists on the mouse isolated stomach assay. *Br. J. Pharmac.*, **86**, 581–587.

BLACK, J.W., LEFF, P. & SHANKLEY, N.P. (1985b). Pharmacological analysis of the pentagastrin-tiotidine interaction in the mouse stomach. *Br. J. Pharmac.*, **86**, 589–599.

BLACK, J.W. & SHANKLEY N.P. (1985a). The isolated stomach preparation of the mouse: a physiological unit for pharmacological analysis. *Br. J. Pharmac.*, **86**, 571–579.

BLACK, J.W. & SHANKLEY, N.P. (1985b). Pharmacological analysis of muscarinic receptors coupled to oxyntic cell secretion in the mouse stomach. *Br. J. Pharmac.*, **86**, 601–607.

ECKNAUER, R., THOMPSON, W.J., JOHNSON, L.R. & ROSENFELD, G.C. (1980). Isolated parietal cells ($^3$H) QNB binding to putative cholinergic receptors. *Am. J. Physiol.*, **239**, G204–209.

ECKNAUER, R., DIAL, E., THOMPSON, W.J., JOHNSON, L.R. & ROSENFELD, G.C. (1981). Isolated rat gastric parietal cells: cholinergic response and pharmacology. *Life Sci.*, **28**, 609–621.

GIACHETTI, A., HAMMER, R. & MONTAGNA, E. (1982). Muscarinic receptor subtypes and responses to McN-A 343 and pirenzepine. *Br. J. Pharmac.*, **77**, 482P.

HAMMER, R. & GIACHETTI, A. (1984). Selective muscarinic

receptor antagonists. *Trends Pharmac. Sci.*, **2**, 18–20.

KENAKIN, T.P. & BEEK, D. (1980). Is prenalterol (H133/80) really a selective beta 1 adrenoceptor agonist? Tissue selectivity resulting from differences in stimulus-response relationships. *J. Pharmac. exp. Ther.*, **213**, 406–413.

LEFF, P., MARTIN, G. & MORSE, J. (1985). Application of the operational model of agonism to establish conditions when functional antagonism may be used to estimate agonist dissociation constants. *Br. J. Pharmac.*, **85**, 655–665.

LONDONG, W. & LONDONG, V. (1982). Pirenzepine for peptic ulcer. In *Receptor Update*, pp. 114–130. Geneva: Excerpta Medica.

MACINTOSH, F.C. (1938). Histamine as a normal stimulant of gastric secretion. *Q.J. exp. Physiol.*, **28**, 87–89.

ODORI, Y. & MAGEE, D.F. (1969). The action of some agents active at autonomic ganglionic sites on the secretory response of the Heidenhain pouch to various stimuli. *Eur. J. Pharmac.*, **8**, 221–227.

ROSZKOWSKI, A.P. (1961). An unusual type of sympathetic ganglionic stimulant. *J. Pharmac.*, **132**, 156–170.

SCHOLES, P.A., COOPER, A., JONES, D., MAJOR, J., WALTERS, M. & WILD, C. (1976). Characterisation of an adenylate cyclase system sensitive to histamine $H_2$-receptor excitation in cells from dog gastric mucosa. *Agents and Actions*, **6**, 677–687.

SOLL, A.H. (1980). Secretogogue stimulation of ($^{14}C$) aminopyrine accumulation by isolated parietal cells. *Am. J. Physiol.*, **238**, G366–375.

SOLL, A.H. (1981). Physiology of isolated parietal cells. In *Physiology of the gastrointestinal tract*, Vol. I, ed. Johnson, L.R., pp. 673–691. New York: Raven Press.

TEPPERMAN, B.L., SCHOFIELD, B. & TEPPERMAN, F.S. (1975). Effect of metiamide on acid secretion from isolated kitten fundic mucosa. *Can. J. Physiol. Pharmac.*, **53**, 1141–1146.

VAN ROSSUM, J.M. (1962). Classification and molecular pharmacology of ganglionic blocking agents. *Int. J. Neuropharmac.*, **2**, 97–110.

WASTEK, G.J. & YAMAMURA, H.I. (1978). Biochemical characterization of the muscarinic cholinergic receptor in human brain: alterations in Huntington's disease. *Mol. Pharmac.*, **14**, 768–780.

YAMAMURA, H.I. & SNYDER, S.H. (1974). Muscarinic cholinergic receptor binding in the longitudinal muscle of the guinea-pig ileum with ($^{3}H$)quinuclidinyl benzilate. *Mol. Pharmac.*, **10**, 861–867.

(*Received March 4, 1985.*
*Revised June 28, 1985.*
*Accepted July 8, 1985.*)