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Characterization of muscarinic receptor and β-adrenoceptor interactions in guinea-pig oesophageal muscularis mucosae

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Abstract

Smooth muscles of a number of species contain both muscarinic M_2 and M_3 receptors in differing proportions and while muscarinic M_3 receptors mediate contraction, the role of muscarinic M_2 receptors is unclear. Muscarinic M_2 receptor-mediated inhibition of adenylyl cyclase activity has been demonstrated in smooth muscle and since β-adrenoceptor relaxation of this tissue is mediated via stimulation of adenylyl cyclase, an interaction between muscarinic M_2 receptors and β-adrenoceptors in smooth muscle has been postulated. Such an interaction has been demonstated in guinea-pig ileum and trachea using two different approaches. The present study investigates whether interactions between muscarinic M_2 receptors and β-adrenoceptors also occur in guinea-pig oesophageal muscularis mucosae. Using the technique of selective muscarinic M_3 receptor alkylation, we were unable to demonstrate muscarinic M_2 receptor-mediated re-contractions in oesophageal smooth muscle, as described previously in ileum. In addition, while increased functional antagonism of relaxant responses to isoprenaline could be demonstrated in tissues pre-contracted with oxotremorine M compared to histamine, muscarinic M_2 receptor activation did not contribute to this effect, as described previously in trachea. These data suggest a lack of interaction between muscarinic M_2 receptors and β-adrenoceptors in guinea-pig oesophageal smooth muscle, but suggest an interaction between muscarinic M_3 receptors and β-adrenoceptors.

Keywords: Muscarinic receptor; Esophageal muscularis mucosae; Receptor alkylation

1. Introduction

Muscarinic receptor heterogeneity in smooth muscle preparations was initially revealed when discrepancies were observed between antagonists affinities, estimated by radioligand binding techniques and functional studies (Roffel et al., 1988). Heterogeneity of smooth muscle muscarinic receptors is now well documented but the roles of each subtype are unclear (see Eglen et al., 1994 for review). Muscarinic M_3 receptors are known to mediate smooth muscle contraction but the role of muscarinic M_2 receptors, often the predominant muscarinic receptor population, is currently the subject of intensive investigation. The observation that activation of muscarinic M_2 receptors in smooth muscle inhibits adenylyl cyclase activity has prompted suggestions that muscarinic M_2 receptors may regulate smooth muscle tone via an inhibitory effect on β-adrenoceptor-mediated relaxations (see Eglen et al., 1994 for review). In guinea-pig ileal smooth muscle an indirect contractile response to muscarinic M_2 receptor stimulation has been demonstrated after selective muscarinic M_3 receptor alkylation and stimulation of adenylyl cyclase (Thomas et al., 1993; Reddy et al., 1995). This contraction, or more correctly termed 're-contraction', is the result of muscarinic M_2 receptor-mediated inhibition of a β-adrenoceptor-mediated relaxation. By contrast, in guinea-pig trachea, while it has not been possible to demonstrate muscarinic M_2 receptor-mediated re-contraction after muscarinic M_3 receptor alkylation, antagonism of muscarinic M_2 receptors augments isoprenaline-induced relaxation of tissues pre-contracted with muscarinic agonists (Watson and Eglen, 1994; Watson et al., 1995a).

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Heterogeneity of smooth muscle muscarinic receptors has been described for trachea and ileum of a number of species (see Eglen et al., 1994 for references). While little data are currently available as to the nature of post-junctional muscarinic receptors in guinea-pig oesophageal muscularis mucosea (Eglen and Whiting, 1988), the smooth muscle nature and anatomical proximity of the oesophagus to both trachea and ileum raises the possibility that it too exhibits muscarinic receptor heterogeneity. Therefore, the aim of the present study was to determine whether an interaction between muscarinic M_2 receptors and β-adrenoceptors can be demonstrated in guinea-pig oesophageal smooth muscle. The two different approaches previously described in ileal and tracheal smooth muscle were used: (i) an indirect procedure, characterising 're-contractions' following selective muscarinic M_3 receptor alkylation (Thomas et al., 1993; Reddy et al., 1995) and (ii) a direct procedure, characterising the role of muscarinic M_2 receptors in isoprenaline-induced relaxations of muscarinic agonist-induced tone (Watson and Eglen, 1994). Binding and biochemical analysis was not undertaken in these studies.

2. Materials and methods

2.1. Tissue preparation

Male Dunkin-Hartley guinea-pigs (250–350 g) were killed by CO_2 exposure and the oesophagus removed along with the trachea down to the level of the diaphragm. The oesophagus was then separated from the trachea and the outer striated muscle coat was carefully removed. The remaining longitudinal muscularis mucosae formed a tube approximately 1.5 cm in length, which was then suspended under 1 g resting tension in 10 ml organ baths containing oxygenated (95% O_2; 5% CO_2) modified Krebs buffer (composition (mM); NaCl 118.2, KCl 4.6, $MgSO_47H_2O$ 1.2, KH_2PO_4 1.2, glucose, 10.0, $NaHCO_3$ 24.8 and $CaCl_2$ 2.5; pH 7.4, 37°C). Indomethacin (1 μM) and tetrodotoxin (0.1 μM) were present to inhibit prostaglandin synthesis and to eliminate possible pre-junctional effects of muscarinic agonists, respectively. Corticosterone (30 μM) was present to inhibit extraneuronal monoamine uptake. Preparations were equilibrated for 60 min before beginning experimental protocols. Concentration-effect curves were performed in a cumulative manner using incremental concentrations spaced at half $\log_{10}$ intervals.

2.2. Responses to oxotremorine M before and after muscarinic M_3 receptor alkylation

The muscarinic receptor alkylating agent, 4-diphenylacetoxy-*N*-(2-chloroethyl) piperidine) (4-DAMP mustard), was used in conjunction with muscarinic M_2 receptor protection by methoctramine (1 μM), to selectively inactivate muscarinic M_3 receptors while leaving muscarinic M_2 receptors functionally intact. Cumulative concentration-effect curves to oxotremorine M, a non-selective, highly efficacious agonist, were compared before and after the alkylation procedure in order to determine the extent of muscarinic M_3 receptor inactivation (Thomas et al., 1993; Reddy et al., 1995). The muscarinic M_3 receptor nature of the subtype mediating contractions after this procedure was confirmed by determining the apparent affinity of *para*-fluoro-hexahydrosiladifenidol (*p*-F-HHSiD).

Concentration-effect curves to oxotremorine M were performed at the start of the experiment to establish control responses. Tissues were then washed and re-equilibrated in the presence of 1 μM methoctramine for 20 min before and then during a 60 min exposure to 40 nM 4-DAMP mustard. After this time, tissues were washed twice at 10 min intervals in the presence of 1 μM methoctramine, to wash out the 4-DAMP mustard while maintaining muscarinic M_2 receptor protection. After this, tissues were washed at 10 min intervals for a further 60 min to eliminate both the methoctramine and remaining 4-DAMP mustard. Following this, concentration-effect curves to oxotremorine M were repeated in the absence or presence of 0.3 μM *p*-F-HHSiD.

2.3. Responses to oxotremorine M before and after muscarinic M_3 receptor alkylation in the presence of histamine and isoprenaline

After selective alkylation of muscarinic M_3 receptors as described above, tissues were exposed to histamine (0.3 μM) and isoprenaline (0.6 μM) before performing cumulative concentration-effect curves to oxotremorine M. This provided the necessary conditions for activation of muscarinic M_2 receptors to cause a re-contraction, by reversal of the relaxant effect of isoprenaline on the histamine contracture (Thomas et al., 1993; Reddy et al., 1995). The nature of the receptor subtype mediating the re-contractions after this procedure was investigated by determining the apparent affinity of methoctramine (1 μM, a concentration blocking 98% of muscarinic M_2 receptors; Melchiorre et al., 1993).

2.4. Responses to oxotremorine M in the presence of histamine and isoprenaline but absence of muscarinic M_3 receptor alkylation

These studies were performed in tissues not exposed to 4-DAMP mustard, in order to demonstrate that muscarinic M_2 receptor-mediated re-contraction is dependent on inactivation of muscarinic M_3 receptors by

alkylation (Thomas et al., 1993; Reddy et al., 1995). However, in the absence of a muscarinic M_2 receptor-mediated re-contraction in alkylated tissues these data controlled for the functional antagonism of contractile responses to oxotremorine M, by the exposure to histamine (0.3 μM) and isoprenaline (0.6 μM). This was necessary to establish that the concentration of isoprenaline chosen was sufficient to reverse the contraction induced by histamine without additionally depressing responses to oxotremorine M.

Tissues were treated exactly as in Section 2.3 above, but were not exposed to 4-DAMP mustard.

2.5. Effect of oxotremorine M and histamine on the relaxant potency of isoprenaline

These studies were performed to determine whether the relaxant potency of isoprenaline was reduced in tissues pre-contracted with oxotremorine M as compared to histamine and whether activation of muscarinic M_2 receptors contributes to this increased functional antagonism.

Concentration-effect curves to oxotremorine M and histamine were established to determine the maximum response and the concentration of each agonist required to increase isometric tension to approximately the same level. Tissues were then washed and re-equilibrated for 60 min in the absence or presence of methoctramine (0.3 μM). The resting tension was then raised by the addition of either oxotremorine M (0.3 μM) or histamine (3 μM) and once a stable contracture was achieved concentration-effect curves to isoprenaline (0.1 nM–1 μM) were performed.

2.6. Measurement and analysis of results

Tissue responses were recorded as changes in isometric tension (mg) using Grass FT04 force displacement transducers and were displayed on a Grass 79B polygraph recorder. Agonist potencies (pD_2) were calculated according to the relationship of Parker and Waud (1971) using a non-linear iterative curve fitting procedure (Kaleidagraph, Synergy Software, Reading, PA, USA). Antagonist apparent affinities (pK_B) were estimated using a single concentration of antagonist (Furchgott, 1972).

Statistically significant differences were assessed by paired and unpaired Student's t-test where appropriate, with $P < 0.05$ being considered significant. All values quoted are the mean ± S.E.M. of data from six animals unless otherwise stated.

2.7. Materials

Methoctramine, p-F-HHSiD (*para*-fluoro-hexahydrosiladifenidol), 4-DAMP mustard (4-diphenylacetoxy-*N*-(2-chloroethyl) piperidine), oxotremorine M and histamine were obtained from Research Biochemicals (Natick, MA, USA). Tetrodotoxin, indomethacin, corticosterone, isoprenaline and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Tetrodotoxin was prepared as a 1.0 mM solution in 0.01 M acetic acid. p-F-HHSiD was prepared as a 10 mM stock solution in ethanol and dilutions were made using distilled water. Indomethacin was prepared as a 1 mg ml^{-1} solution in propylene glycol and solubilized by a brief period (2–3 min) of sonication. Corticosterone was prepared as a 0.1 M solution in dimethyl sulphoxide. All other solutions were prepared using distilled water. 4-DAMP mustard was acidified with dilute acetic acid following solubilization in distilled water. Ascorbic acid (22 μM) was added to solutions of histamine and isoprenaline as an anti-oxidant and these solutions were kept on ice for the duration of the experiments.

3. Results

3.1. Responses to oxotremorine M before and after muscarinic M_3 receptor alkylation

Before alkylation oxotremorine M caused concentration-related contractions of oesophageal smooth muscle preparations with a potency of 7.23 ± 0.06 (Fig. 1). After selective muscarinic M_3 receptor alkylation, using 4-DAMP mustard in conjunction with 1 μM methoctramine (to protect muscarinic M_2 receptors) there was a 60 ± 18-fold rightward shift in the concentration-effect curve to oxotremorine M accompanied by a significant 35 ± 3% decrease in the maximum response. Subsequent addition of p-F-HHSiD (0.3 μM)

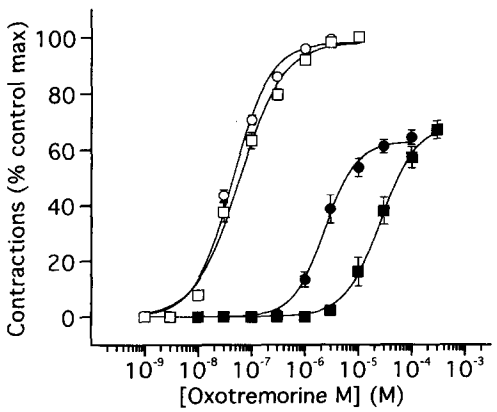

Fig. 1. Concentration-effect curves to oxotremorine M before (open symbols) and after selective muscarinic M_3 receptor alkylation, in the absence (●) and presence (■) of p-F-HHSiD (0.3 μM). Contractions are expressed as a percentage of the maximum control response and are the mean ± S.E.M., $n = 6$.

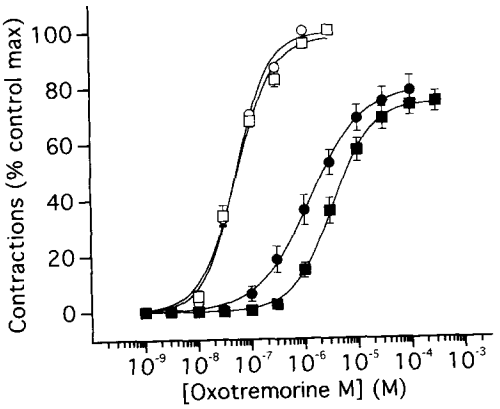

Fig. 2. Concentration-effect curves to oxotremorine M before (open symbols) and after selective muscarinic M_3 receptor alkylation, in the presence of histamine (0.3 μM) and isoprenaline (0.6 μM) with (■) and without (●) methoctramine (1 μM). Contractions are expressed as a percentage of the maximum control response and are the mean ± S.E.M., $n = 6$.

caused an additional rightward shift with an estimated apparent affinity (pK_B) of 7.40 ± 0.13 (Fig. 1).

3.2. Responses to oxotremorine M before and after muscarinic M_3 receptor alkylation in the presence of histamine and isoprenaline

After selective alkylation of muscarinic M_3 receptors, tissues were contracted with histamine (0.3 μM) and maximally relaxed with isoprenaline (0.6 μM) before performing cumulative concentration-effect curves to oxotremorine M. Under these conditions there was a 26 ± 6-fold rightward shift in the concentration-effect curves to oxotremorine M and a 15 ± 4% depression in the maximum response. Methoctramine (1 μM) caused an additional rightward shift in the concentration-effect curve to oxotremorine M with an estimated apparent affinity (pK_B) of 6.18 ± 0.11 (Fig. 2).

3.3. Responses to oxotremorine M in the presence of histamine and isoprenaline but absence of muscarinic M_3 receptor alkylation

In tissues not exposed to 4-DAMP mustard, precontracting with histamine (0.3 μM) and then maximally relaxing with isoprenaline (0.6 μM), prior to performing concentration-effect curves to oxotremorine M, caused a 3-fold rightward shift in the concentration-effect curve to oxotremorine M without significantly affecting the maximum response (Fig. 3). Subsequent addition of methoctramine (1 μM) caused a small additional shift in the concentration-effect curve to oxotremorine M with an estimated affinity of 5.59 ± 0.19.

3.4. Effect of oxotremorine M and histamine on the relaxant potency of isoprenaline

Oxotremorine M and histamine caused concentration-dependent contractions of oesophageal smooth muscle with potencies (pD_2) and maximum contractions of 7.11 ± 0.05, 957 ± 239 mg and 5.92 ± 0.07, 755 ± 122 mg, respectively. The relaxant potency of isoprenaline, in tissues pre-contracted using 3 μM histamine, was greater than in tissues pre-contracted to approximately the same level of isometric tension using 0.3 μM oxotremorine M ($pD_2 = 8.32 \pm 0.11$ and 7.87 ± 0.10, respectively). Methoctramine (0.3 μM) had no significant effect on the relaxant potency of isoprenaline in tissues pre-contracted with either histamine or oxotremorine M ($pD_2 = 8.44 \pm 0.09$ and 7.99 ± 0.13, respectively; Fig. 4) and isoprenaline-induced relaxations in histamine pre-contracted tissues remained significantly greater than in oxotremorine M pre-contracted tissues.

With the exception of tissues pre-contracted with histamine alone (368 ± 62 mg), the magnitude of the developed tensions prior to performing concentration-effect curves to isoprenaline was not significantly different between treatment groups (578 ± 46 mg oxotremorine M alone, 549 ± 103 mg oxotremorine M with methoctramine and 397 ± 126 mg histamine with methoctramine, $n = 7$ for each group). However, when the contractions induced by oxotremorine M and histamine were compared as a percentage of the maximum response to agonist in each tissue there was no significant difference between the four treatment group

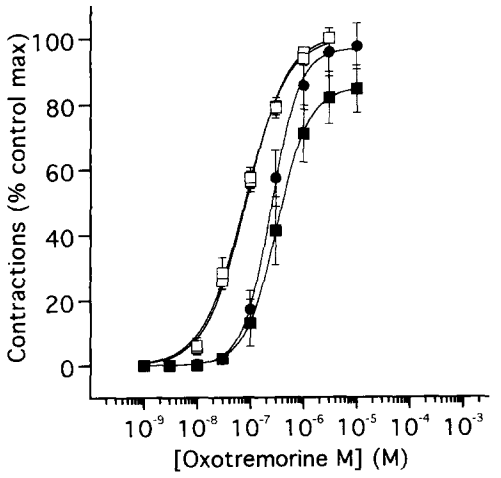

Fig. 3. Concentration-effect curves to oxotremorine M in the absence (open symbols) and presence (filled symbols) of histamine (0.3 μM) and isoprenaline (0.6 μM) with (■) and without (●) methoctramine (1 μM). Contractions are expressed as a percentage of the maximum control response and are the mean ± S.E.M., $n = 6$.

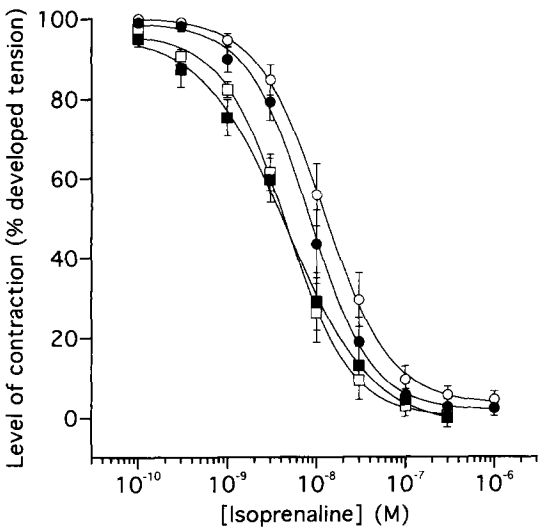

Fig. 4. Isoprenaline-induced relaxation of guinea-pig oesophageal smooth muscle pre-contracted with either oxotremorine M (circles) or histamine (squares) in the absence (open symbols) and presence (closed symbols) of 0.3 μM methoctramine. The level of contraction is expressed as a percentage of the developed tension induced by either oxotremorine M (0.3 μM) or histamine (3 μM). The relaxant potencies and levels of developed tension for all four groups are given in Section 3.4. Maximum responses to isoprenaline were: in oxotremorine M-pre-contracted tissues 539 ± 104 with (●) and 545 ± 43 mg without (○) methoctramine and in histamine-pre-contracted tissues 397 ± 126 with (■) and 368 ± 62 mg without (□) methoctramine. Data are the mean ± S.E.M., $n = 7$ per treatment group.

(63 ± 2% oxotremorine M alone, 59 ± 4% oxotremorine M with methoctramine, 56 ± 6% histamine alone and 55 ± 4% histamine with methoctramine.

4. Discussion

The aim of the present studies was to investigate whether an interaction between muscarinic M_2 receptors and β-adrenoceptors can be demonstrated in guinea-pig oesophageal smooth muscle, as has been demonstrated in ileal and tracheal smooth muscle of this species using two different approaches (Thomas et al., 1993; Reddy et al., 1995; Watson and Eglen, 1994).

An inhibitory role for muscarinic M_2 receptors on β-adrenoceptor function has been demonstrated in guinea-pig ileum after selective muscarinic M_3 receptor alkylation (Thomas et al., 1993; Reddy et al., 1995). Muscarinic M_3 receptor alkylation using the irreversible muscarinic M_3 receptor antagonist, 4-DAMP mustard (Barlow et al., 1990), in conjunction with muscarinic M_2 receptor protection by methoctramine (1 μM), resulted in selective irreversible alkylation of muscarinic M_3 receptors while leaving muscarinic M_2 receptors functionally intact (Thomas et al., 1993; Reddy et al., 1995). Under the conditions used in the present study, a 60-fold rightward shift in the concentration-effect curve to oxotremorine M was observed with a significant depression in maximum responses. This rightward shift was greater than achieved in guinea-pig ileum or trachea under similar conditions (16-fold shift, Reddy et al., 1995; 40-fold shift, Watson et al., 1995a) and may reflect increased affinity of this antagonist for oesophageal muscarinic M_3 receptors, or a lower muscarinic M_3 receptor reserve for contraction in this tissue.

Differences in muscarinic M_3 receptor affinity between smooth muscle preparations have been demonstrated with other muscarinic M_3 receptor antagonists, notably *p*-F-HHSiD (Eglen et al., 1990; Roffel et al., 1994) and zamifenacin (Wallis, 1995; Watson et al., 1995b). The depression in maximum responses to oxotremorine M after alkylation in oesophagus, which was not observed in ileum or trachea under these alkylation conditions (Thomas et al., 1993; Reddy et al., 1995), points to a lower receptor reserve associated with contraction in oesophagus. Additionally the somewhat lower potency of oxotremorine M in oesophagus ($pD_2 = 7.23 \pm 0.06$) compared to ileum ($pD_2 = 7.7 \pm 0.1$, Reddy et al., 1995) and trachea ($pD_2 = 7.41 \pm 0.04$, Watson et al., 1995a) may also suggest a lower receptor reserve for contraction in oesophageal smooth muscle. However, the additional parallel rightward shift in the concentration-effect curve to oxotremorine M produced by *p*-F-HHSiD and the estimated apparent affinity value of 7.4, suggest that a significant proportion of muscarinic M_3 receptors remained to mediate this contraction. This is consistent with findings in both ileum and trachea (Reddy et al., 1995; Watson et al., 1995a).

To reveal muscarinic M_2 receptor-mediated re-contractions after muscarinic M_3 receptor alkylation, tissues were exposed to histamine and isoprenaline (Thomas et al., 1993; Reddy et al., 1995). Under these conditions the concentration-effect curve to oxotremorine M was shifted to the right 26-fold and the depression in the maximum response was reduced to 15%. However, methoctramine (1 μM) caused an additional rightward shift in the response, with an affinity estimate ($pK_B = 6.2$) once again consistent with activation of muscarinic M_3 receptors (6.0, Eglen and Whiting, 1988) and not muscarinic M_2 receptors. This is in contrast to observation in guinea-pig ileal smooth muscle (Thomas et al., 1993; Reddy et al., 1995) where muscarinic M_2 receptors mediate re-contraction, but resembles observations in tracheal smooth muscle under these conditions (Watson et al., 1995a).

In the absence of muscarinic M_3 receptor alkylation, contractile responses to oxotremorine M were shifted to the right 3-fold by the presence of histamine and isoprenaline. These data suggest that the concentration of isoprenaline used (0.6 μM) reversed the

contractile response to histamine (0.3 μM) and also caused a small degree of functional antagonism of contractile responses to oxotremorine M without depressing the maximum response. The conditions for muscarinic M_2 receptor-mediated re-contractions, via inhibition of β-adrenoceptor-mediated relaxation, should therefore be met by these concentrations of histamine and isoprenaline (Thomas et al., 1993; Reddy et al., 1995). Although no muscarinic M_2 receptor-mediated re-contraction could be demonstrated under these conditions, it is interesting to note that the rightward shift in responses to oxotremorine M after muscarinic M_3 receptor alkylation, when histamine and isoprenaline were present, was reduced (26-fold versus 60-fold). The reasons for this are unclear, but similar observations were made in guinea-pig trachea under these conditions (Watson et al., 1995a). Taken together these data may suggest that with the number of functional muscarinic M_3 receptor reduced by alkylation, stimulation of inositol phosphate production by histamine lowers the threshold for M_3-mediated contractions.

The 4-DAMP mustard/methoctramine and histamine/isoprenaline concentrations reported here for oesophagus are identical to those reported for ileum (Reddy et al., 1995), but differ from those in trachea (Watson et al., 1995a). Nevertheless, it could be argued that conditions which were optimal in ileum might not be optimal in oesophagus. Additional experiments were performed in the present study using a lower concentration of methoctramine (0.3 μM) to protect muscarinic M_2 receptors during alkylation and different concentrations of histamine and isoprenaline (30 μM and 0.3 μM, respectively). However, the degree of muscarinic M_3 receptor protection associated with the lower concentration of methoctramine was not significantly different from that seen with the higher concentration, which provided better muscarinic M_2 receptor protection ($33 \pm 3\%$ vs. $35 \pm 5\%$ depression in oxotremorine M max. with 0.3 μM and 1 μM methoctramine, respectively). In the presence of 30 μM histamine and 0.3 μM isoprenaline, marked functional antagonism of oxotremorine M responses was observed ($53 \pm 5\%$ depression in oxotremorine M max. in the absence of alkylation and $80 \pm 4\%$ after alkylation), hence, the conditions considered to be optimal in ileum were found also to be optimal in oesophagus.

An interaction between muscarinic M_2 receptors and β-adrenoceptors was also investigated using the more direct approach, comparing the relaxant potency of isoprenaline in tissues precontracted with oxotremorine M or histamine in the absence and presence of muscarinic M_2 receptor antagonism by methoctramine (Watson and Eglen, 1994). These results show that the relaxant potency of isoprenaline was slightly, but significantly, reduced in tissues pre-contracted with oxotremorine M when compared to histamine, consistent with observations in guinea-pig trachea. However, unlike observations in guinea-pig trachea (Watson et al., 1995a), muscarinic M_2 receptor antagonism by methoctramine (0.3 μM) did not significantly increase the relaxant potency of isoprenaline in tissues pre-contracted with oxotremorine M.

Taken together, these studies indicate a lack of muscarinic M_2 receptor-mediated inhibition of relaxant responses to β-adrenoceptor agonists. It might be argued that the increased relaxant potency of isoprenaline in histamine pre-contracted tissues compared to oxotremorine M pre-contracted tissues was due to the lower level of the developed tension in histamine-pre-contracted tissues in the absence of methoctramine. However, this is unlikely to be the case since the isoprenaline-relaxant potency remained significantly greater in histamine-pre-contracted tissues with methoctramine present, compared to oxotremorine M-pre-contracted tissues both in the absence and presence of methoctramine. In these tissues there was no significant difference in the magnitude of the developed tension before performing cumulative concentration-effect curves to isoprenaline. Additionally, if developed tensions were compared as a percentage of each tissue's own maximum to agonist, then there was no significant difference between the four treatment groups.

The findings from both the alkylation studies and those directly investigating isoprenaline-induced relaxations suggest that an interation between muscarinic M_2 receptors and β-adrenoceptors is unlikely in guinea-pig oesophageal smooth muscle. However, the relaxant potency of isoprenaline in oesophageal smooth muscle is dependent upon the agonist inducing tone, an observation which is consistent with findings in guinea-pig tracheal smooth muscle (Torphy, 1984; Watson and Eglen, 1994) and human bronchial smooth muscle (Watson et al., 1995c). In guinea-pig tracheal smooth muscle activation of muscarinic M_2 receptors contributes in a small way to this effect (Watson and Eglen, 1994; Watson et al., 1995a). However, in oesophageal smooth muscle as with human bronchial smooth muscle (Watson et al., 1995c), no such muscarinic M_2 receptor involvement could be demonstrated.

There are a number of possible explanations for this finding. The difference in potency of isoprenaline in oesophageal smooth muscle pre-contracted via muscarinic or histamine receptors may be due to differences in the level of phosphoinositide metabolism induced by oxotremorine M and histamine. Such a mechanism has been shown to account for a large proportion of the functional antagonism of β-adrenergic relaxations in bovine tracheal smooth muscle (Van Amsterdam et al., 1989; Meurs et al., 1993). Additionally, in the absence of binding data demonstrating heteroge-

neous muscarinic receptor populations in oesophageal smooth muscle, it is possible that the number of muscarinic M_2 receptors in this tissue is small or non-existent. Were this to be the case, then this tissue might prove useful in determining the role of phosphoinositide metabolism in the functional antagonism of β-adrenoceptor-mediated relaxations.

It is concluded that, while muscarinic M_2 receptors have been shown to play an important role in the regulation of guinea-pig ileal smooth muscle tone (Thomas et al., 1993; Reddy et al., 1995) their role in the regulation of guinea-pig oesophageal smooth muscle tone is small, if any. However, the demonstration of increased functional antagonism of β-adrenoceptor-mediated relaxations by a muscarinic agonist in this tissue suggests that an interaction between muscarinic M_3 receptors and β-adrenoceptors is likely to occur. This tissue may therefore provide a useful model for investigating the role of muscarinic M_3 receptors in the functional antagonism of β-adrenoceptor-mediated relaxations.

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