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Short communication

Antagonism of β-adrenoceptor-mediated relaxations of human bronchial smooth muscle by carbachol

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Abstract

Activation of muscarinic M_2 receptors has been suggested to account, in part, for the reduced relaxant potency of β-adrenoceptor agonists in canine and guinea-pig tracheal smooth muscle pre-contracted with muscarinic agonists as compared to histamine. The aim of the present study was to determine whether the potency of isoprenaline is reduced in human bronchial ring preparations pre-contracted with carbachol as compared to histamine and whether activation of muscarinic M_2 receptors contributes to this effect. Cumulative concentration-effect curves to isoprenaline were obtained in the absence and presence of muscarinic M_2 receptor antagonism by methoctramine (0.3 μM) in bronchial ring preparations pre-contracted to equivalent isometric tensions with either histamine (10 μM) or carbachol (1 μM). The relaxant potency of isoprenaline was reduced in preparations pre-contracted with carbachol compared to histamine, but there was no significant effect of muscarinic M_2 receptor antagonism on either the potency or maximal relaxation by isoprenaline. In conclusion, increased functional antagonism of β-adrenoceptor-mediated relaxation by muscarinic agonists can be demonstrated in human bronchial smooth muscle, but muscarinic M_2 receptors do not appear to contribute to this effect.

Keywords: Muscarinic M_2 receptor; β-Adrenoceptor-mediated relaxation; Smooth muscle, human, bronchial

1. Introduction

Airway smooth muscle of a number of species, including man, contains mixed populations of muscarinic M_2 and M_3 receptors (Roffel et al., 1988; Haddad et al., 1991; Mak et al., 1992; Widdop et al., 1993). Stimulation of muscarinic M_3 receptors activates phospholipase C, via a pertussis toxin insensitive G protein to cause contraction. Stimulation of muscarinic M_2 receptors inhibits adenylyl cyclase activity via a pertussis toxin sensitive G protein, but the functional consequences of this are unclear (Caulfield, 1994). In canine and guinea-pig tracheal smooth muscle pre-contracted with muscarinic agonists, the relaxant potency of β-adrenoceptor agonists is increased by antagonism of muscarinic M_2 receptors (Fernandes et al., 1992; Watson and Eglen, 1994) and in canine trachea by pertussis toxin pretreatment (Mitchell et al., 1993). The implication from these findings is that post-junctional muscarinic M_2 receptors may serve to limit β-adrenoceptor-mediated relaxations under conditions of cholinergic tone. The present study investigates whether a similar mechanism may operate in human bronchial smooth muscle, since mRNA for muscarinic M_2 receptors has been demonstrated in human airway smooth muscle (Mak et al., 1992) and muscarinic M_2 receptor mediated inhibition of adenylyl cyclase has been demonstrated in cultured human airway smooth muscle cells (Widdop et al., 1993).

2. Materials and methods

Bronchial tissue was obtained at surgery for lung cancer from 7 patients (6 male, 1 female, mean age = 65; range = 41–70). None was chronically treated with anticholinergic drugs, steroids or theophylline; one patient was taking inhaled β-adrenoceptor agonist on demand. Small bronchi with an internal diameter of

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2–4 mm were dissected from the tissue and placed in oxygenated (95% O_2 : 5% CO_2) modified Krebs buffer (composition (mM): NaCl, 118.4, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1). Tissue was kept at 4°C in oxygenated modified Krebs and used the next day.

Ring segments, 2–3 mm in length, were prepared and mounted in organ baths containing oxygenated modified Krebs (pH 7.4, 37°C) at a resting tension of 250–300 mg. After equilibration for at least 60 min a cumulative concentration-effect curve to either histamine (0.1 μM–0.3 mM) or carbachol (10 nM–30 μM) was performed. Tissues were then washed and re-equilibrated for a further 60 min in the presence or absence of methoctramine (0.3 μM). A single concentration of histamine and carbachol, which gave approximately equal increases in isometric tension but which caused between 60 and 70% maximal contraction (as determined from the initial concentration-effect curves), was then selected, the reason being that at low concentrations of contractile agonist the functional antagonism of isoprenaline-induced relaxations is less marked (Van Amsterdam et al., 1989; Watson and Eglen, 1994). These concentrations of histamine (10 μM) or carbachol (1 μM) were then applied to the appropriate tissues and once stable contractions were attained cumulative concentration-effect curves to isoprenaline were performed (0.1 nM–10 μM).

2.1. Measurement and analysis of results

Responses were recorded as changes in isometric tension (mg). The level of contraction was expressed as a percentage of the isometric tension induced by agonist before application of isoprenaline. In some instances this value fell below 0% since there is a varying amount of spontaneous tone associated with preparations of human bronchial smooth muscle, which is believed to result from the production of cysteinyl-leukotrienes (Rabe et al., 1993; Ellis and Undem, 1994). Data were analysed using a non-linear iterative curve fitting procedure from which potency (pD_2) and maximal responses were determined. Statistical analysis of the data was performed using Student's *t*-tests with $P < 0.05$ being considered significant.

2.2. Materials

Isoprenaline, histamine and carbachol were obtained from Sigma Chemical Company (Deisenhofen, Germany). Methoctramine was obtained from RBI (Natick, MA, USA). All drugs were dissolved in distilled water.

3. Results

Histamine and carbachol produced concentration-related increases in isometric tension with potency (pD_2) and maximal contractions of 5.3 ± 0.1; 1048 ± 81 mg and 6.4 ± 0.1; 1171 ± 88 mg, respectively. Submaximal contractions induced by histamine (10 μM) and carbachol (1 μM) were not significantly different from one another and were not significantly influenced by the presence of 0.3 μM methoctramine (Fig. 1). Isoprenaline caused concentration-dependent relaxations of both histamine- and carbachol-induced contractures (Fig. 1). The relaxant potency of isoprenaline was sig-

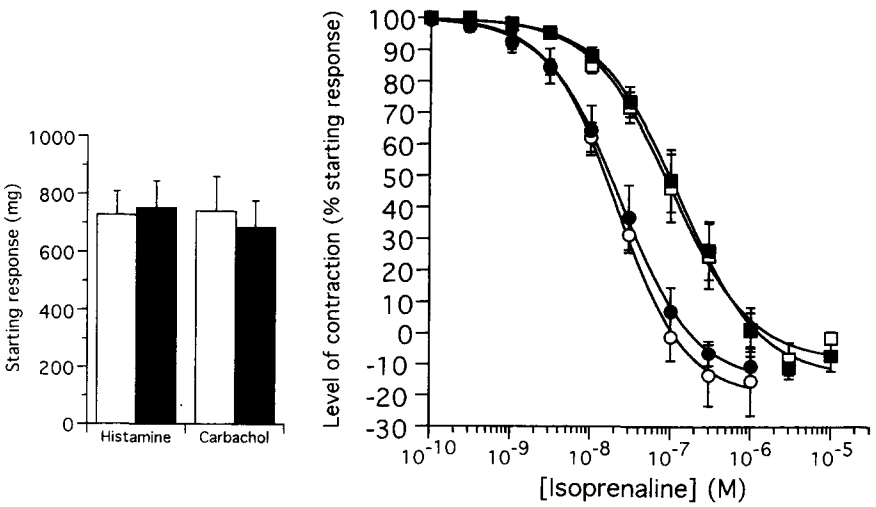

Fig. 1. Isoprenaline-induced relaxations of human bronchial smooth muscle pre-contracted with histamine (circles) or carbachol (squares), in the absence (open symbols) and presence (closed symbols) of M_2 receptor antagonism with methoctramine (0.3 μM). The level of contraction is expressed as a percentage of the starting responses induced by either histamine (10 μM) or carbachol (1 μM) which are shown in absolute values in the bar graph on the left. Open bars represent the response in the absence of methoctramine and filled bars the response in the presence of methoctramine (0.3 μM). Data are the mean ± S.E.M from seven individuals in each of the four treatment groups.

nificantly higher in tissues pre-contracted with histamine ($pD_2 = 7.7 \pm 0.1$) when compared to those pre-contracted to equivalent levels of isometric tension with carbachol ($pD_2 = 6.9 \pm 0.2$). The relaxant potency of isoprenaline was not significantly altered by the presence of 0.3 μM methoctramine, whether tissues were pre-contracted with histamine or carbachol (7.6 ± 0.1 and 6.8 ± 0.2, respectively). The level of isometric tension induced by either histamine or carbachol was not significantly different between the four treatment groups (Fig. 1).

4. Discussion

The present study shows increased functional antagonism of relaxant responses to isoprenaline in human bronchial smooth muscle pre-contracted with the muscarinic agonist carbachol as compared to histamine. These findings are not consistent with previous findings in human bronchial smooth muscle (Van Amsterdam et al., 1990); however, they are consistent with previous reports in canine, rabbit and guinea-pig airway smooth muscle (Fernandes et al., 1992; Arjona et al., 1993; Van Amsterdam et al., 1989; Watson and Eglen, 1994). Methodological differences are unlikely to account for the difference observed by Van Amsterdam and colleagues (1990), since the same group (Van Amsterdam et al., 1989) has previously demonstrated reduced isoprenaline relaxant potency in guinea-pig tracheal smooth muscle pre-contracted with methacholine compared to histamine, using similar methodology to that reported for human bronchial smooth muscle (Van Amsterdam et al., 1990). A possible explanation for this difference may be the more robust activation of phospholipase C by carbachol compared to methacholine (Van Amsterdam et al., 1989).

In canine and rabbit tracheal smooth muscle, muscarinic M_2 receptors contribute to the increased functional antagonism seen with muscarinic agonists (Fernandes et al., 1992; Arjona et al., 1993). One study in guinea-pig tracheal smooth muscle reports a similar involvement of muscarinic M_2 receptors in the increased functional antagonism seen with the muscarinic agonist (+)-*cis*-dioxolane (Watson and Eglen, 1994), while another demonstrated no involvement of muscarinic M_2 receptors in the increased functional antagonism seen with methacholine (Roffel et al., 1993). The increased functional antagonism seen here with carbachol in human bronchial smooth muscle does not appear to involve activation of muscarinic M_2 receptors, since there was no effect of muscarinic M_2 receptor antagonism with methoctramine, in agreement with the latter study in guinea-pig (Roffel et al., 1993).

Based on apparent antagonist affinity values (pK_B) of 7.8 and 6.3 for methoctramine at muscarinic M_2 and M_3 receptors, respectively (Melchiorre et al., 1993), 95% and 37% of these receptors would be occupied by 0.3 μM methoctramine and 98% and 67% by 1 μM methoctramine. Since our objective was to determine whether activation of muscarinic M_2 receptors contributed to the reduced relaxant potency of isoprenaline, 0.3 μM methoctramine was used because of the near maximal M_2 receptor occupancy and negligible M_3 receptor occupancy associated with this concentration. Higher concentrations of methoctramine, being associated with increased muscarinic M_3 receptor antagonist activity, reduce the contractile responses to 1 μM carbachol (data not shown) making interpretation of the data difficult. Therefore, these data demonstrate for the first time in vitro, reduced relaxant potency of isoprenaline in human bronchial smooth muscle pre-contracted with carbachol compared to histamine. This effect is independent of muscarinic M_2 receptor activation and may depend on the ability of the contractile agonist to induce phosphoinositide turnover (Van Amsterdam et al., 1990).

The reasons for the species difference, with respect to the muscarinic M_2 receptor involvement, are unclear but may reflect differences in muscarinic M_2 receptor number and/or efficiency of coupling to the functional response. Canine tracheal smooth muscle contains up to 90% muscarinic M_2 receptors (Fernandes et al., 1992), while only 50–60% of total muscarinic receptors in guinea-pig tracheal smooth muscle are of the M_2 subtype (Haddad et al., 1991). The reduced number of muscarinic M_2 receptors in guinea-pig trachea compared to canine trachea and the observation that in guinea-pig trachea these receptors may be poorly coupled to the inhibition of relaxant responses to isoprenaline (Watson and Eglen, 1994), may account for the mixed reports of muscarinic M_2 receptor involvement in antagonism of β-adrenoceptor-mediated relaxations in this species (Roffel et al., 1993; Watson and Eglen, 1994).

Information regarding the relative proportions of muscarinic receptors in human bronchial smooth muscle is not yet available, although muscarinic inhibition of adenylyl cyclase activity in cultured human airway smooth muscle cells has been reported (Widdop et al., 1993). The inhibitory effect in these cells is due to the activation of muscarinic M_2 receptors, since pertussis toxin treatment reversed the effect and the antagonist apparent affinity profile was consistent with activation of muscarinic M_2 receptors. It should be noted, however, that these were cultured cells from human trachealis muscle and, therefore, the same may not be true of bronchial smooth muscle in vitro. Additionally, the fact that a significant muscarinic M_2 receptor population could not be demonstrated in human lung by either binding or autoradiographic techniques (Mak

and Barnes, 1989, 1990), may suggest that the proportion of M_2 receptor in human airways is small.

In light of the lack of data regarding the relative proportions of M_2/M_3 receptors in human airways, studies are required to address this issue and to further characterise the muscarinic receptor subtypes in human airways and their involvement in the functional antagonism of β-adrenoceptor-mediated relaxations.

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