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β_2- but not β_3-adrenoceptors mediate prejunctional inhibition of non-adrenergic non-cholinergic contraction of guinea pig main bronchi

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Abstract

We studied the effects of selective β-adrenoceptor agonists on the cholinergic and non-adrenergic non-cholinergic (excitatory NANC) contractions elicited by electrical field stimulation of guinea pig main bronchi in vitro. Addition of the selective β_2-adrenoceptor agonists, fenoterol and salbutamol, and the selective β_3-adrenoceptor agonist, BRL 37344 (4-[2-[(2-hydroxy-2-(3-chlor-phenyl)ethyl)amino]-propyl]-phenoxyacetic acid), induced a dose-dependent inhibition of the cholinergic contraction (pD_2 7.89, 6.71 and 4.56, respectively) and the excitatory NANC response (pD_2 9.11, 8.16 and 7.42, respectively). Fenoterol- and BRL 37344-induced inhibition of the excitatory NANC response was blocked with high potency (pK_B 8.77 and 9.07, respectively) by the selective β_2-adrenoceptor antagonist, ICI 118,511 (erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamino)-butan-2-ol). A comparable contraction induced by neurokinin A (2 or 5 nM) was also inhibited by fenoterol, salbutamol and BRL 37344, but at significantly higher concentrations than for the inhibition of the excitatory NANC response (pD_2 8.72, 7.56 and 6.66, respectively). Such a preferential inhibition of electrical field stimulation- versus agonist-induced effects was not observed for cholinergic contractions (pD_2 versus methacholine-induced tone 7.86, 6.93 and 5.10, respectively). The results clearly exclude the involvement of β_3-adrenoceptors in these responses. Furthermore they show that β_2-adrenoceptors are involved in the prejunctional inhibition of excitatory NANC contractions, presumably via modulation of tachykinin release from sensory nerves, and solely in the postjunctional inhibition of cholinergic contractions.

Keywords: β_2-Adrenoceptor; β_3-Adrenoceptor; Electrical field stimulation; Cholinergic contraction; NANC (non-adrenergic non-cholinergic) contraction; Bronchus, main, guinea pig

1. Introduction

The autonomic nervous system controls many aspects of airway function. In addition to the classical cholinergic and adrenergic mechanisms, there is a third component of neural control, which is neither adrenergic nor cholinergic. This non-adrenergic, non-cholinergic (NANC) innervation can exert either contractile (excitatory NANC) or relaxant (inhibitory NANC) actions on the smooth muscle (Barnes, 1986). The excitatory NANC response is mediated by the release of neuropeptides from non-myelinated sensory nerves. The peptides involved may include the tachykinins, substance P, neurokinin A and neurokinin B (Hua et al., 1985), and calcitonin gene-related peptide (Palmer et al., 1987). They produce their effects by activating tachykinin NK_1, NK_2, NK_3, and calcitonin gene-related peptide receptors, respectively. It has been concluded that mainly tachykinin NK_2 receptors (and possibly some tachykinin NK_1 receptors) are involved in tachykinin-induced contraction of guinea pig isolated bronchi (Regoli et al., 1987), and that they belong to the tachykinin NK_{2A} subtype (Maggi et al., 1991). Several prejunctional receptor systems like α_2-adrenoceptors (Grundström and Andersson, 1985), μ-opioid receptors (Belvisi et al., 1988), $GABA_B$ receptors (Belvisi et al., 1989), histamine H_3 receptors (Ichinose and Barnes, 1989) and neuropeptide Y receptors (Stretton et al., 1990) have been indicated to modulate the release of neuropeptides from sensory nerves. Furthermore, the involvement of prejunctional β-adrenoceptors inhibiting tachykinin release from sensory nerves

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in guinea pig bronchi has been proposed, although the receptor subtype has been a matter of controversy. Thus, it was recently concluded that β_3-adrenoceptors mediate this response, since the rather potent inhibition of excitatory NANC contractions, compared to that of contractile responses to exogenous substance P, by the selective β_3-adrenoreceptor agonist, BRL 37344, and its prodrug, BRL 35135, was resistant to non-selective or selective β_1-adrenoceptor antagonists (Itabashi et al., 1992). Verleden et al. (1993), on the other hand, suggested the involvement of β_2-adrenoceptors, since the preferential effect of selective β_2-adrenoceptor agonists in inhibiting excitatory NANC (compared to exogenous substance P) contraction was largely prevented by propranolol and the selective β_2-adrenoceptor antagonist, ICI 118,551. Recently, Martin et al. (1993) concluded that there was a functional presence of β_3-adrenoceptors on sensory nerve endings, after having shown that the potent inhibition by the selective β_3-adrenoceptor agonists, BRL 37344 and SR 58611A, was only partially affected by propranolol and ICI 118,551; the additional involvement of β_2-adrenoceptors, however, was not excluded.

In an attempt to solve this debate, we have investigated the effects of the β_2-adrenoceptor agonists, fenoterol and salbutamol, and the β_3-adrenoceptor agonist, BRL 37344, on excitatory NANC contractions elicited by electrical field stimulation in vitro, in comparison to the relaxant effects on contractions induced by the exogenous agonist, neurokinin A. BRL 37344 was chosen because this selective β_3-adrenoceptor agonist was recently found to mediate relaxation of rat esophagus smooth muscle (having both β_2- and β_3-adrenoceptors) with high potency and exclusively through the β_3-adrenoceptor population (De Boer et al., 1993). The experiments were performed in the absence and presence of the selective β_2-adrenoceptor antagonist, ICI 118,551. Moreover, the effects of the β_2- and β_3-adrenoceptor agonists on cholinergic twitch contractions and methacholine-induced contractions were studied in order to establish prejunctional β-adrenoceptor-mediated inhibition of cholinergic contractions, which has been suggested (Barnes, 1992) in guinea pig main bronchi.

2. Materials and methods

2.1. Mechanical responses

Adult (450–750 g) outbred guinea pigs of either sex were killed by a sharp blow on the head and exsanguinated. Tracheae and lungs were rapidly removed and placed in Krebs-Henseleit solution (37°C) of the following composition (mM): NaCl 117.5, KCl 5.6, $MgSO_4$ 1.18, $CaCl_2$ 2.5, NaH_2PO_4 1.28, $NaHCO_3$ 25.0, and glucose 5.5, gassed with 5% CO_2 and 95% O_2; pH 7.4. Main bronchi were gently cleaned of connective tissue and single ring preparations were placed between two parallel platinum electrodes in 20.0 ml water-jacketed organ baths (37°C) containing Krebs-Henseleit solution, under isotonic recording conditions using a 300 mg load. Cholinergic contractions and excitatory NANC responses were elicited by electrical field stimulation (square wave pulses of 8 V and 0.5 ms duration at a frequency of 30 Hz; Grass S88 stimulator;

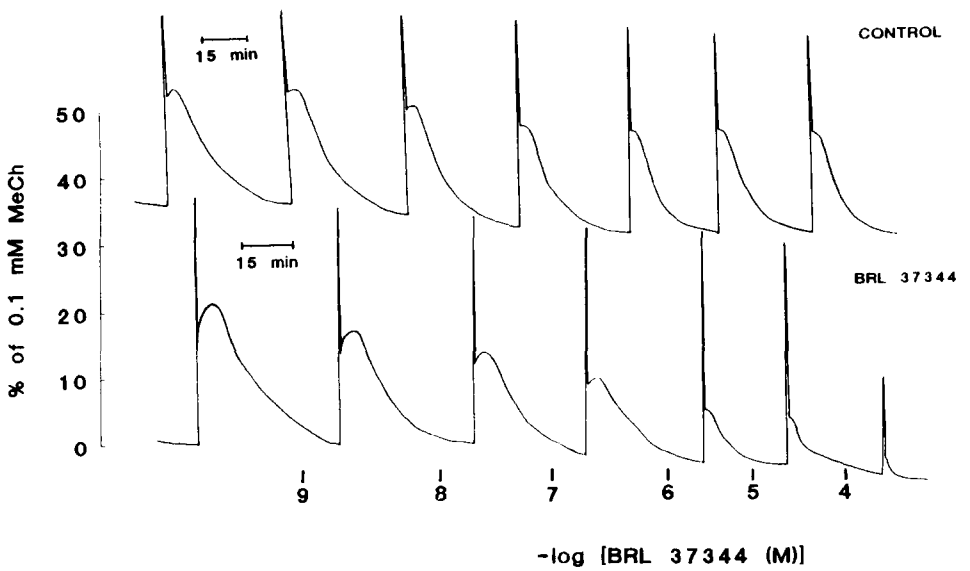

Fig. 1. Typical examples of repeated electrical field stimulation-induced cholinergic and excitatory NANC responses of a guinea pig main bronchial single ring preparation in the absence (upper panel) and presence (lower panel) of increasing concentrations of the selective β_3-adrenoceptor agonist, BRL 37344. The vertical axis refers to both recordings.

Ten Berge et al., 1993) applied for 20 s. In preliminary experiments, these stimulation parameters were found to be optimal for the simultaneous stimulation of cholinergic and non-cholinergic nerve endings to obtain cholinergic and excitatory NANC contractions that were reproducible between different animals.

2.2. Experimental protocol

Following an equilibration period of 30 min the bronchial smooth muscle preparations were relaxed using (−)-isoprenaline (0.1 μM) to establish basal tone. After a washout period of 30 min the maximal contraction was determined with cumulative additions of methacholine (0.1, 1, 10 and 100 μM). The preparations were then washed for 60 min, and electrical field stimulation was started. Indomethacin (3 μM) was continuously present to eliminate any influence of the generation of cyclooxygenase products. Stimulation evoked a rapid cholinergic twitch contraction followed by a second, prolonged contraction, the excitatory NANC response, which lasted for 15–20 min (Fig. 1). Electrical field stimulation was performed 7–8 times on each preparation, with 30 min between two consecutive stimulations.

The effects of the selective β_2-adrenoceptor agonists, fenoterol and salbutamol, and the selective β_3-adrenoceptor agonist, BRL 37344, on the excitatory NANC response were studied in the absence and presence of the selective β_2-adrenoceptor antagonist, ICI 118,551 (0.1 μM). After a control response to electrical field stimulation had been obtained, increasing concentrations of the β-adrenoceptor agonists were added 10 min prior to each subsequent stimulation. ICI 118,551 was added 15 min before stimulation. As a control, repeated electrical field stimulation was carried out in the absence of any drug in parallel preparations. The effects of fenoterol, salbutamol and BRL 37344 on exogenously induced contractile responses were also evaluated by cumulative addition of the β-adrenoceptor agonists; care was taken that each concentration was applied after the effect of the preceding concentration had reached equilibrium. To obtain contraction levels with exogenous agonists that were comparable to those induced by electrical field stimulation, neurokinin A (2 or 5 nM) and methacholine (10 μM) were used.

2.3. Drugs

Methacholine chloride, (−)-isoprenaline hydrochloride, capsaicin, salbutamol hemisulfate and tetrodotoxin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Atropine sulfate was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Fenoterol hydrobromide (Boehringer Ingelheim, Germany), indomethacin (Merck Sharp & Dohme, Haarlem, Netherlands), BRL 37344 (4-[2-[(2-hydroxy-2-(3-chlor-phenyl)ethyl)amino]-propyl]-phenoxyacetic acid;

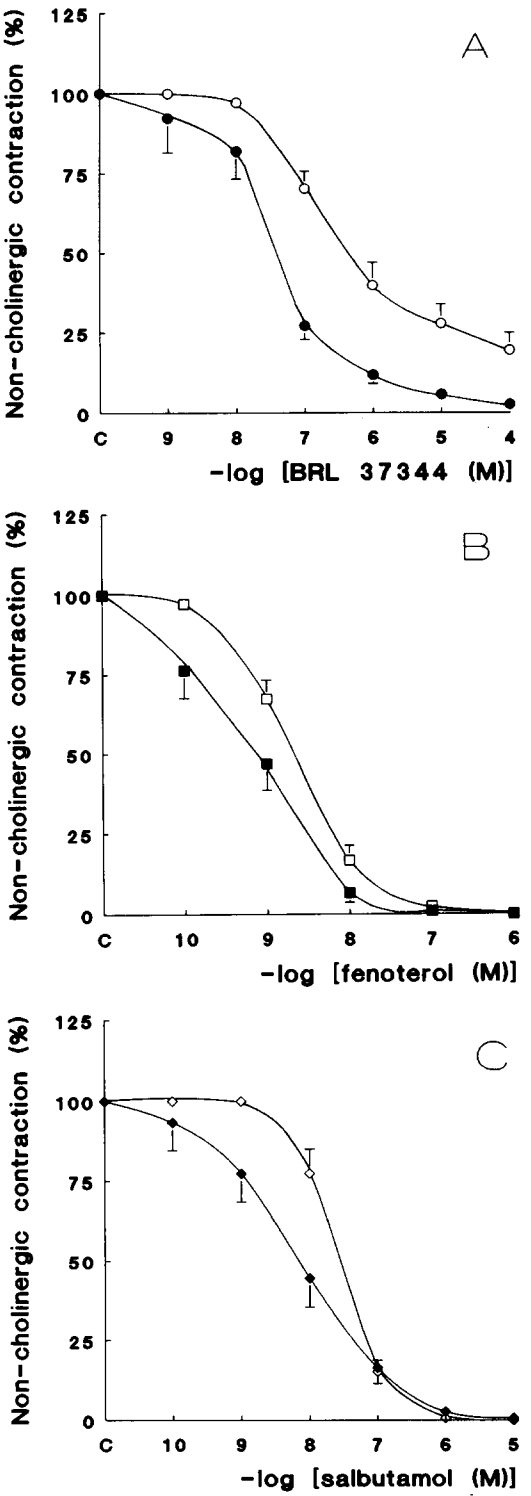

Fig. 2. Inhibition of electrical field stimulation-induced excitatory NANC (closed symbols) and neurokinin A-induced (open symbols) contractions by the selective β_3-adrenoceptor agonist, BRL 37344 (A), and the selective β_2-adrenoceptor agonists, fenoterol (B) and salbutamol (C). The data represent means ± S.E.M. of 3–12 experiments as indicated in Table 1.

SmithKline Beecham, Epsom, UK) and ICI 118,551 (erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol; ICI, Macclesfield, UK) were kindly provided by the manufacturers.

2.4. Data analysis

The excitatory NANC and cholinergic contractions elicited by electrical field stimulation in the presence of β-adrenoceptor agonists were expressed as a percentage of the control response. The excitatory NANC response was estimated as area under the contraction curve, the electrical field stimulation-induced cholinergic response as peak height of the twitch contraction. Dose-inhibition curves with excitatory NANC responses were corrected for the decline that was observed in the parallel control responses (Fig. 1). The contraction level induced by exogenous agonists was expressed as a percentage of maximal contraction achieved with 100 μM methacholine. Antagonist pK_B values were calculated according to: $pK_B = -\log\{[\text{antagonist}]/(DR - 1)\}$. The data were expressed as means ± S.E.M. Statistical differences were evaluated using a two-tailed Student's t-test. Significance was accepted at $P < 0.05$.

3. Results

The contractile responses elicited by electrical field stimulation (8 V, 30 Hz, 0.5 ms for 20 s) were completely abolished by tetrodotoxin (1 μM), confirming the neural nature of the response (not shown). Similarly, atropine (1 μM) and capsaicin (30 μM) pretreatment eliminated the electrical field stimulation-induced cholinergic and excitatory NANC contractions, respectively, assuring the cholinergic and non-adrenergic non-cholinergic origin of these contractile responses.

Table 1
pD_2 values of BRL 37344, fenoterol and salbutamol for the inhibition of the electrical field stimulation-induced excitatory NANC response and for relaxation of the neurokinin A-induced contraction

	Excitatory NANC		Neurokinin A	
	pD_2	Control contraction level (%)	pD_2	Control contraction level (%)
BRL 37344	7.42±0.10	21.5±3.4 (12)	6.66±0.08 [a]	22.1±3.4 (7)
Fenoterol	9.11±0.13	18.6±2.8 (11)	8.72±0.09 [b]	22.9±2.9 (8)
Salbutamol	8.16±0.13	25.0±7.0 (5)	7.56±0.09 [b]	19.2±1.6 (3)

Control contraction levels of the excitatory NANC and neurokinin A-induced contractions (2 or 5 nM) are expressed as percentages of those with 0.1 mM methacholine. Data are shown as means±S.E.M. (n determinations). Significant differences compared to excitatory NANC: [a] $P < 0.001$, [a] $P < 0.05$.

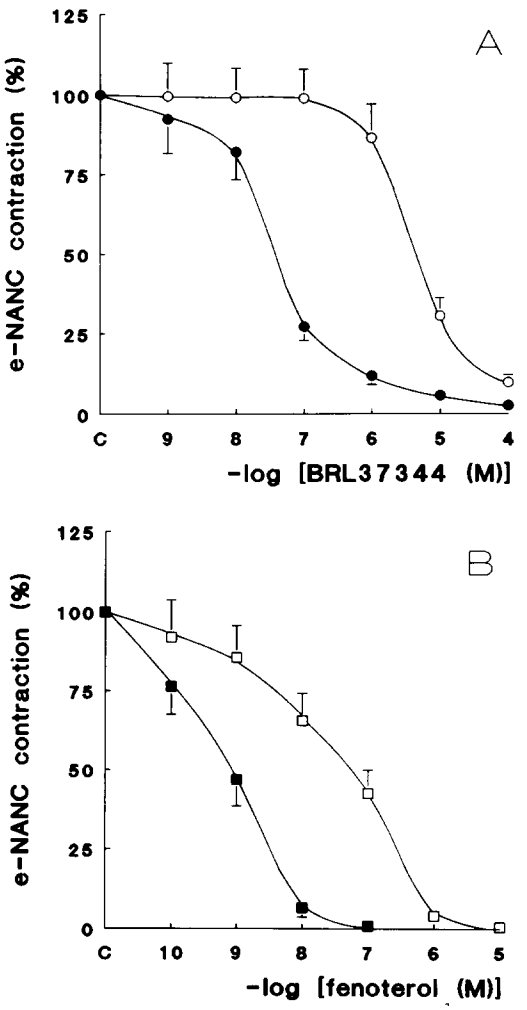

Fig. 3. Inhibition of electrical field stimulation-induced excitatory NANC contractions by the selective β_3-adrenoceptor agonist, BRL 37344 (A), and the selective β_2-adrenoceptor agonist, fenoterol (B), in the absence (closed symbols) and presence (open symbols) of the selective β_2-adrenoceptor antagonist, ICI 118,551 (0.1 μM). The data represent means±S.E.M. of 8–12 experiments.

3.1. Effects of the selective β_2- and β_3-adrenoceptor agonists on the electrical field stimulation-induced excitatory NANC and neurokinin A-induced contractions

Both the selective β_3-adrenoceptor agonist, BRL 37344 (Fig. 2A), and the selective β_2-adrenoceptor agonists, fenoterol (Fig. 2B) and salbutamol (Fig. 2C), produced a concentration-dependent and complete inhibition of the electrical field stimulation-induced excitatory NANC responses, yielding pD_2 values as shown in Table 1. The effects of BRL 37344 and fenoterol on the excitatory NANC responses were blocked with high potency by ICI 118,551 (0.1 μM) (Figs. 1 and 3), yielding pK_B values of 9.07 and 8.77, respectively.

Fig. 2 shows the inhibitory effects of the β-adrenoceptor agonists on electrical field stimulation-induced excitatory NANC and on neurokinin A-induced con-

tractions. The rank order of potency was fenoterol > salbutamol > BRL 37344 for both types of contraction (Table 1). However, contractions induced by exogenous

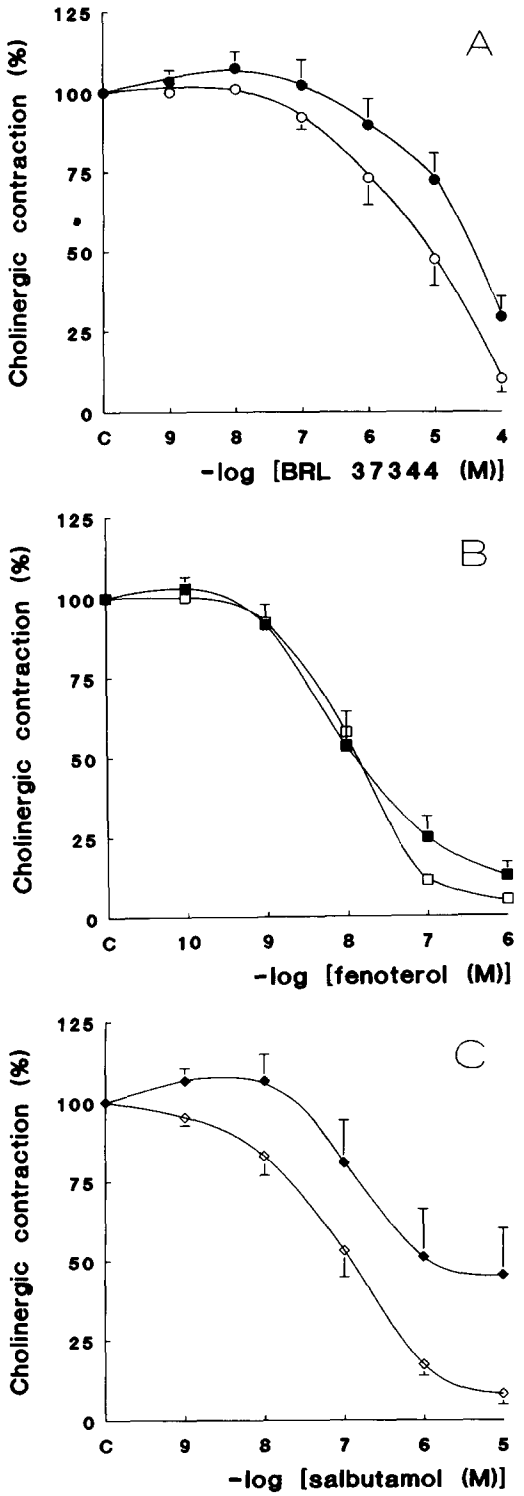

Fig. 4. Inhibition of electrical field stimulation-induced cholinergic contractions (closed symbols) and methacholine-induced contractions (open symbols) by the selective β_3-adrenoceptor agonist, BRL 37344 (A), and the selective β_2-adrenoceptor agonists, fenoterol (B) and salbutamol (C). The data represent means ± S.E.M. of 4–12 experiments as indicated in Table 2.

Table 2
pD_2 values of BRL 37344, fenoterol and salbutamol for the inhibition of the electrical field stimulation-induced cholinergic contraction and relaxation of methacholine-induced (1 μM) contractions

	Electrical field stimulation		Methacholine	
	pD_2	Control contraction level (%)	pD_2	Control contraction level (%)
BRL 37344	4.56 ± 0.17	36.7 ± 3.7 (12)	5.10 ± 0.24	44.4 ± 6.8 (4)
Fenoterol	7.94 ± 0.09	40.6 ± 4.2 (11)	7.86 ± 0.05	50.7 ± 5.1 (5)
Salbutamol	6.84 ± 0.20	35.7 ± 7.8 (5)	6.93 ± 0.13	49.4 ± 2.3 (6)

Contraction levels are expressed as percentages of those with 0.1 mM methacholine. Data are shown as means ± S.E.M. (n determinations).

neurokinin A, applied at equi-contractile concentrations compared to the height of the excitatory NANC response (19–25% of maximal methacholine contraction), were inhibited at significantly higher concentrations of all agonists than were the excitatory NANC responses (Table 1).

3.2. Effects of the selective β_2- and β_3-adrenoceptor agonists on the electrical field stimulation-induced cholinergic and methacholine-induced contractions

Fenoterol-, salbutamol- and BRL 37344-induced relaxations of electrical field stimulation-induced cholinergic and methacholine-induced contractions of comparable level are shown in Fig. 4; pD_2 values and contraction levels are given in Table 2. The rank order of potency for both types of relaxation was fenoterol > salbutamol > BRL 37344, identical to the inhibition of non-cholinergic contractions (Table 1). However, methacholine-induced contractions were relaxed at equal fenoterol concentrations and even at lower concentrations (not significantly different) of salbutamol and BRL 37344, compared to electrical field stimulation-induced cholinergic contractions (Table 2).

4. Discussion

The present study was designed to investigate the putative role of β_2- and β_3-adrenoceptors in the pre- and postjunctional modulation of electrical field stimulation-induced cholinergic and excitatory NANC neural contractions in guinea pig isolated main bronchi. To accomplish this, we compared the effects of BRL 37344, which is selective for β_3-adrenoceptors (Arch et al., 1984), to those of the selective β_2-adrenoceptor agonists, fenoterol and salbutamol.

4.1. Effects on electrical field stimulation-induced excitatory NANC responses

Our study showed that both the β_3- and the β_2-adrenoceptor agonists were able to inhibit the electri-

cal field stimulation-induced excitatory NANC contractions with high potency (Table 1), in agreement with previous findings with BRL 37344 and salbutamol. However, in contrast to part of these previous studies (Itabashi et al., 1992; Martin et al., 1993), clear evidence was obtained that the inhibitory response to both fenoterol and BRL 37344 is mediated via typical β_2-adrenoceptors, since the dose-inhibition curves for these compounds were markedly shifted to the right by the selective β_2-adrenoceptor antagonist, ICI 118,551, yielding pK_B values as high as 8.77 and 9.07, respectively. In order to establish whether the β-adrenoceptor agonists elicited their inhibitory effects via pre- and/or postjunctional β_2-adrenoceptors, we compared the effects on excitatory NANC responses to relaxation of neurokinin A-induced contractions. Neurokinin A was chosen as the exogenous agonist, since tachykinin NK_2 receptors are the predominant subtype in tachykinin-induced contraction of guinea pig bronchi (Regoli et al., 1987), and neurokinin A is the major endogenous agonist for these receptors (Maggi et al., 1991). Relaxation of neurokinin A-induced contractions required significantly higher concentrations of BRL 37344, salbutamol and fenoterol than the inhibition of the excitatory NANC response. The higher sensitivity of the electrical field stimulation-induced contractions to these agonists strongly suggests the involvement of prejunctional inhibition of the excitatory NANC response, in addition to postjunctional β_2-adrenoceptor-mediated relaxation. It should be noticed that the difference between agonist potencies to inhibit excitatory NANC- and neurokinin A-induced contractions increases with decreasing β-adrenoceptor agonist potency (Table 1), BRL 37344 even behaving postjunctionally as a partial agonist (Fig. 2A). Since salbutamol has also been reported to have a lower intrinsic activity than fenoterol on tracheal smooth muscle β_2-adrenoceptors (O'Donnell and Wanstall, 1978), the results suggest that inhibition of tachykinin release is more susceptible to prejunctional β_2-adrenoceptor-mediated cAMP generation than relaxation of the neurokinin A-induced contraction is to postjunctional β_2-adrenoceptor-mediated cAMP generation. In other words, prejunctional β_2-adrenoceptors may have a larger receptor reserve than postjunctional β_2-adrenoceptors following stimulation by the same agonist.

Our conclusion of prejunctional β_2-adrenoceptor-mediated inhibition of the excitatory NANC response in guinea pig main bronchi is at variance with some of the previous reports on adrenoceptors modulating this response. Thus, Kamikawa and Shimo (1990) concluded that, in guinea pig isolated bronchial muscle, catecholamines inhibit non-cholinergic excitatory neurotransmission by postjunctional β- and prejunctional α_2-adrenoceptors. In our opinion, however, their results actually suggest the involvement of prejunctional β-adrenoceptors, since isoprenaline (1 μM) was able to inhibit the excitatory NANC response more than the substance P-induced contraction. Itabashi et al. (1992), on the other hand, showed that the selective β_3-adrenoceptor agonist, BRL 37344, and its prodrug, BRL 35135, selectively inhibited excitatory NANC contractions compared to contractile responses to exogenously administered substance P, and suggested the involvement of prejunctional β_3-adrenoceptors. However, these authors did not use selective β_2-adrenoceptor agonists or antagonists, and therefore the involvement of inhibitory β_2-adrenoceptors on sensory nerve endings cannot be excluded. In a study performed by Verleden et al. (1993) the inhibitory effects of the selective β_2-adrenoceptor agonists, aformoterol and salbutamol, were investigated. These agonists inhibited the excitatory NANC responses at significantly lower concentrations than substance P-induced contractions. Furthermore, the effect of aformoterol was largely prevented by the selective β_2-adrenoceptor antagonist, ICI 118,551, while atenolol, a specific β_1-adrenoceptor antagonist, and phentolamine, a non-selective α-adrenoceptor blocking agent, failed to prevent the NANC inhibition by aformoterol, providing evidence that neither β_1- nor α-adrenoceptors are involved in this response. Thus, in agreement with the present results, β_2-adrenoceptors were concluded to inhibit excitatory NANC responses at a prejunctional level (Verleden et al., 1993). However, no selective β_3-adrenoceptor agonists were used in that study, leaving the possibility of (additional) β_3-adrenoceptors still open. Recently, Martin et al. (1993) showed that, whereas the inhibitory effect of salbutamol on excitatory NANC responses was strongly reduced by ICI 118,551, the weak inhibition by the selective β_3-adrenoceptor agonists, BRL 37344 and SR 58611A, was partially or not at all affected by this selective β_2-adrenoceptor antagonist, respectively. They suggested that β_3-adrenoceptors are involved in the prejunctional inhibitory control of electrical field stimulation-induced excitatory NANC responses in guinea pig main bronchi, and that salbutamol acts predominantly on postjunctional β_2-adrenoceptors, although a prejunctional component was not ruled out.

It is difficult to reconcile all the data described above, and to explain the observed differences. In most of the studies, the exogenous neuropeptide contraction was induced with the selective tachykinin NK_1 receptor agonist, substance P, whereas tachykinin NK_2 receptors are mainly involved in neuropeptide contraction in this tissue. In our study, therefore, in order to obtain a better reflection of the electrical field stimulation-induced excitatory NANC response, we applied the more tachykinin-selective NK_2 receptor agonist, neurokinin A. Martin et al. (1993) used both substance P and the tachykinin-selective NK_2 receptor agonist, [Nle^{10}]neu-

rokinin A-(4–10), and did not observe clear differences in inhibitory effects by the β-adrenoceptor agonists. Therefore, the use of different exogenous neuropeptide agonists does not explain the differences in behavior of the selective β-adrenoceptor agonists in the various studies. As an alternative possibility, differences in the method of evaluation of excitatory NANC responses (peak height versus area under the curve), which was not clearly described in the paper by Martin et al. (1993), may influence the outcome of these experiments. However, our data unequivocally indicate the sole involvement of β_2-adrenoceptors in the prejunctional inhibition of the excitatory NANC response in guinea pig main bronchi in vitro.

4.2. Effects on electrical field stimulation-induced cholinergic contractions

In addition to the pre- and postjunctional inhibition of excitatory NANC responses, the β-adrenoceptor agonists investigated also inhibited electrical field stimulation-induced cholinergic twitch contractions, with the same potency order: fenoterol > salbutamol > BRL 37344 (Table 2). In order to establish whether this response is mediated by pre- and/or postjunctional β-adrenoceptors, data derived from electrical field stimulation-induced cholinergic contractions were compared with relaxation data obtained with methacholine as the contractile agonist. It was found that pD_2 values obtained for relaxation of methacholine-induced contractions were identical to (fenoterol; Fig. 4B) or even somewhat higher than those obtained for inhibition of electrical field stimulation-induced contractions (BRL 37344 and salbutamol; Fig. 4A and C; Table 2). These results do not provide any evidence that inhibitory β-adrenoceptors are functionally present on vagal nerve endings in guinea pig central airways, confirming the results of Kamikawa and Shimo (1986), and they suggest that only postjunctional β_2-adrenoceptors are involved in inhibition of cholinergic contractions of guinea pig main bronchus (Zaagsma et al., 1983; Kamikawa and Shimo, 1986,1990; Martin et al., 1993).

Interestingly, the selective β_2-adrenoceptor agonists were about 20 times less potent to inhibit electrical field stimulation-induced cholinergic contractions compared to non-cholinergic electrical field stimulation-induced contractions, and BRL 37344 was even 700-fold less potent on cholinergic contractions. A 10 times higher potency for isoprenaline to inhibit non-cholinergic contractions was similarly shown by Kamikawa and Shimo (1990), and Martin et al. (1993) found 10-fold higher potencies for both BRL 37344 and salbutamol. The lower efficacy of all these agonists might be due to the greater amount of inositol phosphates generated with acetylcholine or methacholine as contractile agonists (Van Amsterdam et al., 1989), which requires more cyclic AMP, and hence higher concentrations of the β-adrenoceptor stimulants, especially the weak agonist BRL 37344, to relax tone.

In conclusion, the present study showed that β_2- but not β_3-adrenoceptors are involved in both pre- and postjunctional inhibition of excitatory NANC responses in guinea pig main bronchi, but that cholinergic contractions are only inhibited by postjunctional β_2-adrenoceptors. It also appears that prejunctional inhibition of (excitatory NANC) contractions, by decreasing transmitter release from nerve endings, requires less cAMP than postjunctional smooth muscle relaxation.

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