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Degradation of acetylcholine in human airways: role of butyrylcholinesterase

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1 Neostigmine and BW284C51 induced concentration-dependent contractions in human isolated bronchial preparations whereas tetraisopropylpyrophosphoramide (iso-OMPA) was inactive on airway resting tone.

2 Neostigmine (0.1 μM) or iso-OMPA (100 μM) increased acetylcholine sensitivity in human isolated bronchial preparations but did not alter methacholine or carbachol concentration-effect curves.

3 In the presence of iso-OMPA (10 μM) the bronchial rings were more sensitive to neostigmine. The pD_2 values were, control: 6.05 ± 0.15 and treated: 6.91 ± 0.14.

4 Neostigmine or iso-OMPA retarded the degradation of acetylcholine when this substrate was exogenously added to human isolated airways. A marked reduction of acetylcholine degradation was observed in the presence of both inhibitors. Exogenous butyrylcholine degradation was prevented by iso-OMPA (10 μM) but not by neostigmine (0.1 μM).

5 These results suggest the presence of butyrylcholinesterase activity in human bronchial muscle and this enzyme may co-regulate the degradation of acetylcholine in this tissue.

Keywords: Human bronchus; butyrylcholinesterase; acetylcholinesterase; acetylcholine; butyrylcholine; tetraisopropylpyrophosphoramide; neostigmine

Introduction

The inherent tone observed in human airways is maintained by the local actions of neuronal inputs derived from the parasympathetic nervous system. This system regulates airway calibre by activation of the post-synaptic muscarinic sites on airway smooth muscle via the release of the neurotransmitter, acetylcholine (ACh). Enzymatic degradation of ACh occurs through the action of cholinesterases. Two different cholinesterases have been described in many tissues (Mann, 1971; Aas *et al.*, 1986; Chatonnet & Lockridge, 1989; Small *et al.*, 1990) namely, acetylcholinesterase (AChE: EC 3.1.1.7) and butyrylcholinesterase (BChE: EC 3.1.1.8). Previous investigators (Mittag *et al.*, 1971; Small *et al.*, 1990; Adler *et al.*, 1991) have shown that BChE represents a considerable fraction of the cholinesterase pool in smooth muscle. While the function of AChE at the cholinergic neuronal synapse in airway muscles is to regulate the duration of action of ACh (Adler & Filbert, 1990), the physiological role of BChE has not been systematically examined in human airways. *In vivo*, treatment with cholinesterase inhibitors leads to an increase in resistance to airflow in animals (Pauluhn *et al.*, 1987) and in man (Haber *et al.*, 1987). Recently, Adler and co-workers (1991) proposed that BChE may participate in the co-regulation of the duration of action of ACh in dog airways *in vitro*. These investigators demonstrated that the contractions induced by electrical field stimulation were enhanced and prolonged in tissues treated with the specific BChE inhibitor, tetraisopropylpyrophosphoramide (iso-OMPA; Atack *et al.*, 1989). While Ito and co-workers (1989) have demonstrated that physostigmine, a non-specific cholinesterase inhibitor, enhanced the response to electrical field stimulation in human isolated bronchial muscles, few studies have been performed to examine the relative contribution of these two enzymes on the functional response of human airways.

The aim of this study was to determine the sensitivity of human bronchial preparations to exogenous and endogenous ACh in the presence of specific cholinesterase inhibitors. A measurement of the degradation of exogenous ACh or butyrylcholine (BuCh: specific substrate for BChE) were also performed in order to compare the activity of AChE and BChE in human isolated bronchial preparations. These studies were undertaken to test the hypothesis that BChE may be a co-regulator of ACh activity in human airways.

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Methods

Isolated preparations

Human lung tissue was obtained from 34 male and 6 female patients who had undergone surgery for lung carcinoma. The mean age was 56.20 ± 1.15 years. After the resection of a lung or a lobe, parts of the bronchus were dissected free from parenchymal lung tissue and placed in Tyrode solution at 4°C for 12 h. All experiments were performed on 161 subsegmental bronchial preparations derived from 40 lung samples (n); these bronchial ring preparations were of 2–4 mm internal diameter and weighed 63.09 ± 2.84 mg. Values are means ± s.e.mean.

Physiological studies (contractions)

Bronchial ring preparations were set up in the 10 ml organ baths containing Tyrode solution (concentration mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4, 0.4 and glucose 5.5; pH 7.4; gassed with 95% O_2/5% CO_2 and maintained at 37°C. The preparations were placed under initial loads (2–3 g) depending on their size.

These loads ensured that responses to contractile agonists were maximal. Isometric force displacement transducers (Narco F-60) and physiographs (Linseis) were used to record the changes in force. The tissues were allowed to equilibrate for 90 min and the bath fluid was exchanged every 15 min with fresh Tyrode solution.

After this period, all bronchial preparations were contracted with ACh (100 μM). The tissues were washed with

fresh Tyrode solution and allowed to return passively to their resting tone. When resting tone was established, two different protocols were followed. In the first protocol, each cholinesterase inhibitor (neostigmine, BW284C51 or iso-OMPA) was added to the bath fluid at 10 min intervals in a cumulative fashion, beginning with the lowest concentration. In the second protocol, the preparations were incubated for 30 min in Tyrode solution containing either neostigmine or iso-OMPA, and subsequently a cholinoceptor agonist-response relationship was determined. Cumulative concentration-effect curves to neostigmine were also produced in the tissues after a 30 min incubation with iso-OMPA. Atropine 1 μM was added 30 min before or at the end of the cholinesterase inhibitors concentration-effect curves.

Biochemical studies (enzyme activities)

Bronchial rings were placed in 10 ml tubes containing 5 ml Tyrode solution gassed with 95% O_2/5% CO_2 and maintained at 37°C. After a 60 min equilibration period, the preparations were pre-incubated for 30 min in Tyrode solution or Tyrode solution containing cholinesterase inhibitors (neostigmine or iso-OMPA) at different concentrations. ACh or BuCh (0.5 μmol) was then added to each tube: 1 h and 3 h after this substrate challenge, a 30 μl sample of supernatant was collected and stored at −20°C. The cholinesterase inhibitors were always present in appropriate tubes during the 3 h following the pre-incubation period. Measurements of choline, ACh and BuCh in the supernatants were performed according to the technique of Israël & Lesbats (1985). This assay involved hydrolysis of ACh by AChE to give choline, which was oxidized to betaine by choline oxidase with H_2O_2 production. Using this technique for measurement of BuCh, the AChE was markedly less efficient in degrading this substrate. In the presence of luminol, H_2O_2 and horseradish peroxidase (HRP), light emission was generated and recorded by a photomultiplier (1250 LKB-Wallac) connected to a potentiometric recorder (2210-032 LKB-Wallac). The assay was carried out in a microcuvette containing 250 μl of phosphate buffer (Na_2HPO_4/NaH_2PO_4 0.2 M; pH 8.6) and 10 μl of a mixture containing choline oxidase (62.5 u ml^{-1}), horseradish peroxidase (0.5 g l^{-1}) and luminol (0.5 mM). The baseline level of luminescence was obtained using these enzymes and buffer. When the samples or choline standards (10 μl) were added to this mixture a peak of luminescence was induced. After the baseline had been re-established, 10 μl of purified AChE (less than 250 u ml^{-1}) was added to determine the ACh or BuCh present in samples or standards. Standards were made up daily, and were tested for each lung sample; the relationship between peak luminometer deflection and amount of added standard in the assay system was linear over the range (4 pmol–2 nmol). Sample values were derived from standard curves. In each lung sample experiment, stock solutions of ACh or BuCh were prepared twice: initially for the tissue incubations in the tubes and again for standards in the chemiluminescence technique.

Data analysis

Physiological studies (contractions) The changes in force were measured from isometric recordings and expressed in grams (g) or as a percentage of the initial contraction induced by ACh (100 μM). The maximal response (E_{max}) was induced with a cholinoceptor agonist, neostigmine or BW284 C51 and the EC_{50} values were interpolated from the individual concentration-effect curves. The EC_{50} values were transformed into pD_2 values, that is, negative logarithms of EC_{50} values.

Biochemical studies (enzyme activities) The amounts of ACh or BuCh remaining in the supernatants after 1 or 3 h as well as the quantities obtained after treatment of tissues with enzyme inhibitors were expressed in μmol. The protection of ACh or BuCh degradation by the different inhibitors was calculated from the following formula: $100 \times (S_c - S_b)/(S_a - S_b)$ where S_a = 0.5 μmol of ACh or BuCh, S_b = the quantity (μmol) of ACh or BuCh remaining after 3 h of incubation with Tyrode solution, S_c = μmol of ACh or BuCh remaining after 3 h of incubation with a cholinesterase inhibitor.

All results are expressed as means ± s.e.mean. Statistical analysis was performed using Multirange ANOVA followed by a post-hoc test (Least Significant Difference) with a confidence level of 95% and taking into account the preparations derived from the same or different lung samples.

Drugs

The drugs and their sources were: acetylcholine chloride, butyrylcholine chloride, choline chloride (three times crystallized), methacholine chloride (acetyl-β-methylcholine chloride), carbachol (carbamylcholine chloride), atropine sulphate, iso-OMPA (tetraisopropylpyrophosphoramide), BW284C51 (1, 5-*bis* (4-allyldimethylammoniumphenyl) pentane-3-one dibromide), Trizma buffer solution [Tris(hydroxymethyl) aminomethane], choline oxidase from *Alcaligenes* species, peroxidase HRP II (Horseradish peroxidase type II) (Sigma Chemical Co., St. Louis, MO, U.S.A.). Neostigmine methylsulphate (Prostigmine; Roche, 92521 Neuilly sur Seine, France). Luminol (5-amino-1,2,3,4 tetrahydrophtalazindion-1,4) (Merck, Schuchardt 8011 Hohenbrunn bei München, Germany). Acetylcholinesterase from *Electrophorus electricus* was obtained from Boehringer Mannheim GmbH (Germany) and purified by passing through a Sephadex G50 coarse column (Pharmacia, Uppsala, Sweden).

All cholinoceptor agonists and BW284C51 were dissolved in Tyrode solution and enzymes were dissolved in distilled water. Iso-OMPA (0.1 M) was dissolved in 100% ethanol. Subsequent dilutions of each drug and neostigmine were made in Tyrode solution. A stock solution of luminol (1 mM) was prepared by dissolving 18 mg in a few drops of 1 M NaOH, and the volume was adjusted to 100 ml with 0.2 M Tris buffer (final pH of 8.6).

Results

Physiological studies (contractions)

The data presented in Figure 1 demonstrate that neostigmine produced concentration-dependent contractions in human isolated bronchial preparations whereas iso-OMPA did not provoke contractions under similar experimental conditions. The pD_2 values for neostigmine and BW284C51 on human bronchial preparations were: 6.46 ± 0.23 and 6.73 ± 0.22, respectively, while the maximal effect of neostigmine was two fold greater than that of BW284C51. These data were obtained in paired preparations derived from 4 lung samples. The neostigmine and BW284C51 responses were completely reversed or inhibited by atropine (1 μM). Pretreatment of human bronchial muscles with neostigmine (0.1 μM; 30 min; Figure 2a and Table 1) or iso-OMPA (100 μM; 30 min; Figure 2b and Table 1) significantly shifted to the left the ACh curves in these preparations without modifying the reactivity (E_{max}). In contrast, these inhibitors at the same concentrations failed to alter the methacholine or carbachol concentration-effect curves (Table 1). Iso-OMPA (10 μM) did not shift ACh curves (Table 1).

The concentration-effect curves obtained with neostigmine were shifted to the left when the bronchial preparations were pretreated with iso-OMPA (30 min; 10 μM; Figure 3 and Table 1).

Biochemical studies (enzyme activities)

Three hours after the addition of exogenous ACh (0.5 μmol) or BuCh (0.5 μmol) to human isolated bronchial ring preparations, a marked reduction in the quantities of these substrates

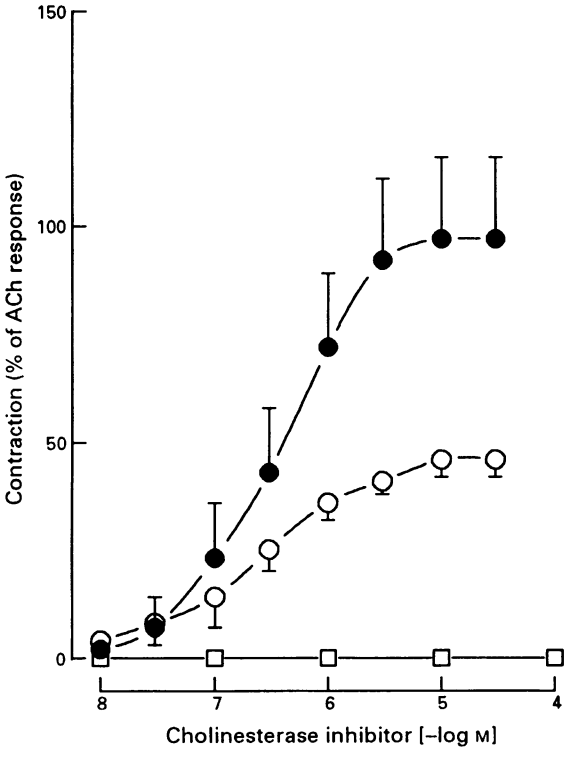

Figure 1 Effect of cholinesterase inhibitors on basal tone in human isolated bronchial preparations. Cumulative concentration-effect curves were constructed with neostigmine (●; $n = 4$), BW284C51 (○, $n = 4$) or tetraisopropylpyrophosphoramide (iso-OMPA, □; $n = 5$). Each response was expressed as a percentage of the acetylcholine (ACh, 100 μM) contraction induced prior to the cumulative-curve. Values are means with s.e.mean shown by vertical bars.

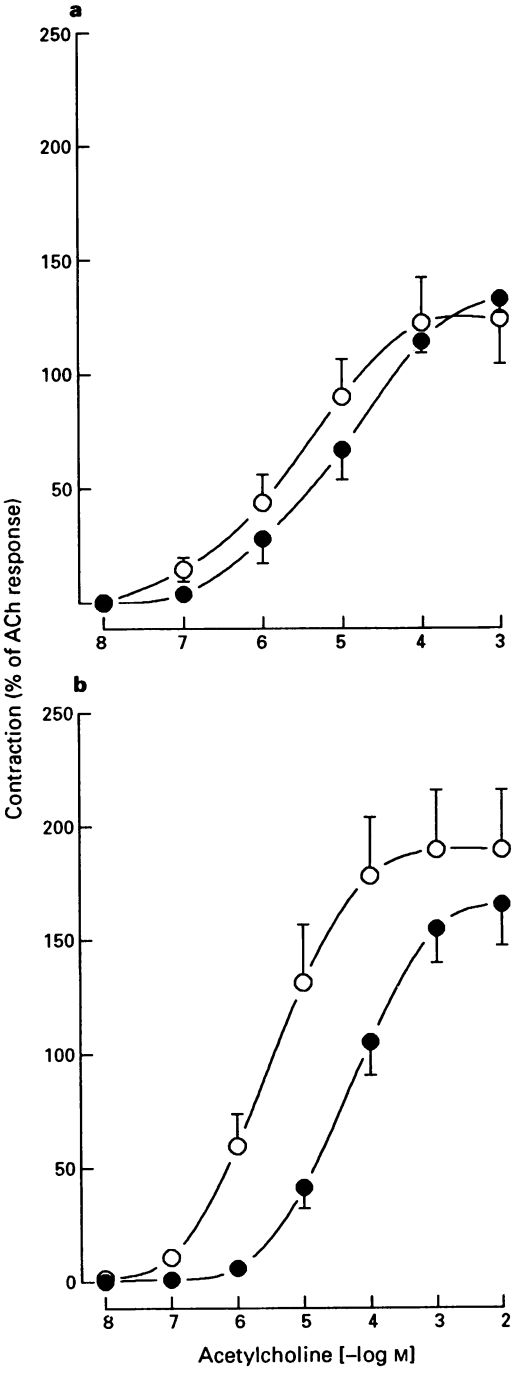

Figure 2 Acetylcholine (ACh) concentration-effect curves produced in human isolated bronchial preparations after 30 min incubation with Tyrode solution (●) or cholinesterase inhibitors (○). (a) Neostigmine (0.1 μM; $n = 6$) (b) tetraisopropylpyrophosphoramide (iso-OMPA, 100 μM; $n = 4$). Each response was expressed as a percentage of the ACh (100 μM) contraction induced prior to the incubation. Values are means ± s.e.mean from paired lung samples.

was observed: ACh (0.10 ± 0.04 μmol; $n = 7$) and BuCh (0.05 ± 0.03 μmol; $n = 4$). This reduction was also detectable at 1 h: ACh (0.25 ± 0.07 μmol; $n = 7$) and BuCh (0.17 ± 0.06 μmol; $n = 4$).

In paired preparations which had been pretreated for 30 min with neostigmine (0.1 μM), prior to the addition of ACh, or in tissues treated with iso-OMPA (10 μM), before the BuCh challenge, a small or no reduction in the quantities of these substrates was detected. Thus at these concentrations, the enzyme inhibitors prevented the hydrolysis of the appropriate substrate (Table 2). In experiments with BuCh, this substrate was degraded even if tissues were treated with neostigmine (Figure 4 and Table 2). The protection of ACh or BuCh by each inhibitor at the concentrations used and neostigmine 10 μM are shown in Table 2. The ACh remaining at 1 h after treatment with neostigmine (0.1 μM) or iso-OMPA (100 μM) were: 0.49 ± 0.05 μmol and 0.50 ± 0.06 μmol, respectively. However, in tissues treated with both neostigmine (0.1 μM) and iso-OMPA (100 μM), the quantity of ACh detected was 0.59 ± 0.05 μmol ($n = 7$ paired lung samples). This quantity was significantly greater than those detected when tissues were treated with the individual inhibitors. The ACh results obtained after 3 h with the inhibitors were: 0.32 ± 0.06 μmol and 0.43 ± 0.06 μmol for neostigmine (0.1 μM) and iso-OMPA (100 μM), respectively. The ACh remaining after 3 h in tissues treated with a combination of both inhibitors at these same concentrations was: 0.51 ± 0.05 μmol. These latter data were greater than results obtained in supernatants in the presence of one inhibitor. When lower concentrations of these enzyme inhibitors were used under the same experimental conditions, a more pronounced alteration was observed, that is, both inhibitors in combination significantly protected ACh against degradation at 1 h (Figure 5a) and at 3 h (Figure 5b).

Choline measurements for each of the preceding experiments were exactly the reverse of all measurements of ACh or BuCh. The choline content increased in supernatants when degradation of ACh or BuCh occurred specifically when tissues were not exposed to cholinesterase inhibitors (data not shown).

Table 1 Effects of cholinesterase inhibitors on acetylcholine, carbachol, methacholine and neostigmine concentration-effect curves in human isolated bronchial preparations

Treatment	n	*ACh* (100 μM) *response* (g)	*Agonist* E_{max} (g)	pD_2 *value*
			Acetylcholine	
Tyrode	17	1.74 ± 0.29	2.42 ± 0.36	4.70 ± 0.36
Iso-OMPA (10 μM)	5	1.42 ± 0.28	2.30 ± 0.55	4.91 ± 0.12
Iso-OMPA (100 μM)	6	1.78 ± 0.48	3.14 ± 0.69	5.57 ± 0.26*
Neostigmine (0.1 μM)	6	2.30 ± 0.56	2.90 ± 0.84	5.68 ± 0.20*
			Carbachol	
Tyrode	4	1.86 ± 0.50	2.88 ± 0.74	6.34 ± 0.03
Iso-OMPA (100 μM)	4	1.90 ± 0.38	2.65 ± 0.55	6.50 ± 0.12
			Methacholine	
Tyrode	3	0.95 ± 0.40	1.22 ± 0.43	6.76 ± 0.03
Neostigmine (0.1 μM)	3	1.47 ± 0.53	1.63 ± 0.49	6.60 ± 0.26
			Neostigmine	
Tyrode	5	1.46 ± 0.38	1.42 ± 0.27	6.05 ± 0.15
Iso-OMPA (10 μM)	5	1.77 ± 0.27	2.41 ± 0.18	6.91 ± 0.14*

Acetylcholine (ACh, 100 μM) response was obtained prior to treatment. Tissues were exposed to Tyrode solution or to Tyrode solution containing cholinesterase inhibitors (30 min). E_{max} indicates the maximal contraction produced by the agonist expressed in grams (g). Values are means ± s.e.mean, *n* indicates the number of lung samples.
*Values significantly different when results from control and treated tissues were compared ($P<0.05$, ANOVA).

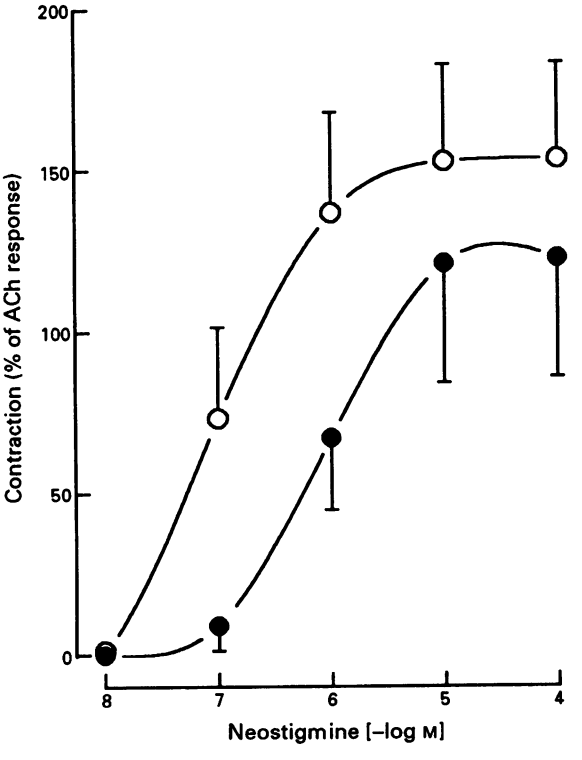

Figure 3 Effect of tetraisopropylpyrophosphoramide (iso-OMPA) on neostigmine concentration-effect curves in human isolated bronchial preparations. Data are derived from curves produced after a 30 min incubation with Tyrode solution (●) or Tyrode solution containing iso-OMPA (10 μM; ○). Each response was expressed as a percentage of the acetylcholine (ACh, 100 μM) contraction induced prior to the incubation. Values are means ± s.e.mean from paired lung samples. Preparations were derived from 5 lungs.

Table 2 Protection against the degradation of acetylcholine (ACh) and butyrylcholine (BuCh) in human isolated bronchial preparations

	Neostigmine (μM) 0.01	0.1	10	*Iso-OMPA* (μM) 10	100
Substrate					
ACh	21 ± 16 (4)	66 ± 17 (7)	102 ± 13 (4)	19 ± 05 (4)	96 ± 22 (7)
BuCh	3 ± 10 (3)	25 ± 04 (4)	52 ± 06 (3)	94 ± 09 (4)	106 ± 10 (7)

Results are expressed as percent protection against the degradation of ACh or BuCh. Protection was evaluated 3 h after the addition of 0.5 μmol of substrate. Values are means with s.e.mean. Number of lung samples used is indicated in parentheses.

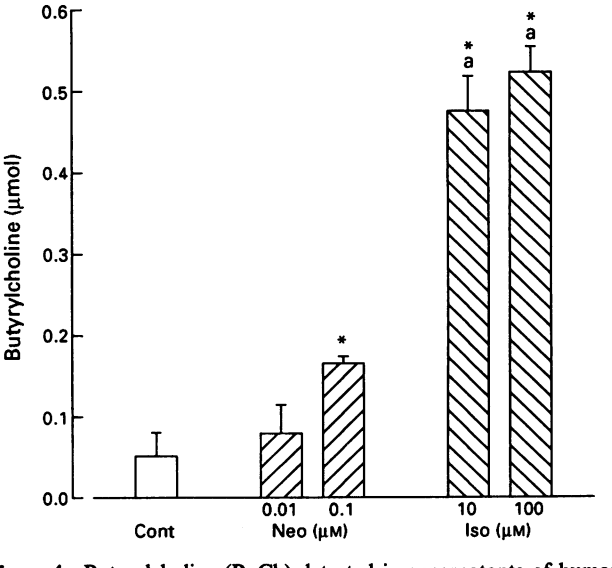

Figure 4 Butyrylcholine (BuCh) detected in supernatants of human isolated bronchial preparations after 3 h incubation with Tyrode solution (Cont) or Tyrode solution containing neostigmine (Neo) or tetraisopropylpyrophosphoramide (Iso). The quantity of BuCh initially added (time zero) was 0.5 μmol. Values are means ± s.e.mean of tissues derived from 3–4 lung samples. Values significantly different from (*) control data or (a) neostigmine (0.1 μM) results ($P<0.05$, ANOVA).

Discussion

The results presented in this paper demonstrate that two functional cholinesterase enzyme pools are present in human bronchial muscle preparations. Hydrolysis of ACh is achieved by the action of endogenous AChE and is inhibited by neostigmine or BW284C51 (Atack *et al.*, 1989; this paper). In addition, BuCh degradation occurs via BChE (Mittag *et al.*, 1971), an activity which is prevented by pretreatment of the tissues

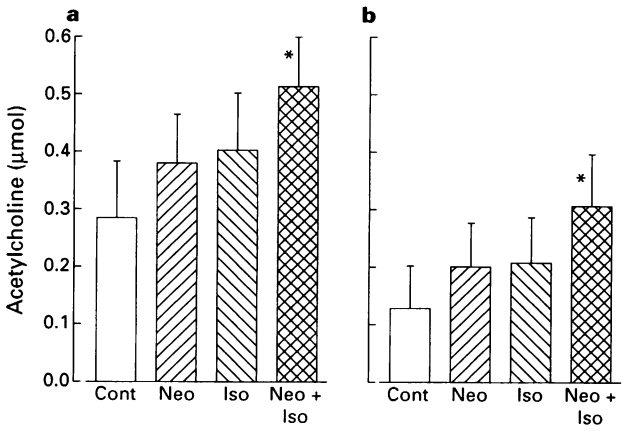

Figure 5 Acetylcholine (ACh) detected in supernatants of human isolated bronchial preparations after 1 h (a) or 3 h incubation (b) in Tyrode solution (Cont) or Tyrode solution containing: neostigmine (Neo; 0.01 μM); tetraisopropylpyrophosphoramide (Iso; 10 μM) or a combination of both inhibitors at these concentrations (Neo + Iso). The quantity of ACh initially added (time zero) was 0.5 μmol. Values shown are means ± s.e.mean from paired lung samples. Preparations were derived from 4 lungs. (*) Values significantly different when compared with the three other values ($P<0.05$, ANOVA).

with iso-OMPA (Austin & Berry, 1953; Thomsen *et al.*, 1991; this paper). In bronchial preparations, the responses induced with BW284C51 were approximately one half of those responses observed with neostigmine (Figure 1). These results support the anti-muscarinic effect of BW284C51 as described by Ambache & Lessin (1955) and Adler *et al.* (1991). Therefore, we used neostigmine as the AChE inhibitor in all other protocols. The data obtained in human airways (this paper) are similar to those results derived from canine tracheal smooth muscle preparations (Adler & Filbert, 1990; Adler *et al.*, 1991). These functional studies support the histochemical findings in which both cholinesterases have been detected in respiratory smooth muscles from a variety of animal species (Mann, 1971; Small *et al.*, 1990) including man (Partanen *et al.*, 1982).

Initial studies dealing with the effects of AChE activity in airway preparations have shown that inhibition of this enzyme by agents such as physostigmine and neostigmine increases the contractile response to exogenous ACh in airway preparations (Douglas, 1951; De Candole *et al.*, 1953; Daly, 1957; Carlyle, 1963) or to electrical field stimulation (Kirkpatrick & Rooney, 1982; Ito *et al.*, 1989). However, none of these reports distinguished between the relative contributions of the different cholinesterases involved in the contractions to cholinoceptor agonists in airway muscles. Recently, Adler and co-workers (1991) demonstrated that both AChE and BChE play a role in the co-regulation of canine airways response to electrical field stimulation. These observations are supported by the results obtained in human airways (this paper), where the role of BChE in the ACh contraction can be observed in tissues which were challenged with increasing concentrations of neostigmine in the presence of iso-OMPA (see Figure 3). These functional data (contractions) are supported by the biochemical measurements in bronchial tissues treated with exogenous ACh in the presence of iso-OMPA (see Figure 5).

While the activities of both enzymes were detected in canine and human isolated airways, few reports have described the distribution of these enzymes in respiratory tissues. The suggestion that these enzymes were distributed differently in different tissues, was based on an early report (Koelle & Koelle, 1959) which showed that AChE in autonomic ganglia of the cat was confined to the neural elements, whereas BChE was restricted to the glial cells. Appleyard & Smith (1989) showed that BChE was present in ileal smooth muscle, whereas AChE was detected in nerves of Auerbach's plexus. Recent evidence derived from the guinea-pig trachea (Small *et al.*, 1990) indicated that BChE was mainly detected in smooth muscle cells while AChE was found in nerve fibres running within the smooth muscle. These data suggest a tissue distribution for the cholinesterases with AChE exhibiting a close association with neuronal elements, while BChE has an extra-neuronal location. Our physiological results (Figure 1) are in agreement with these reports. Muscarinic M_3-receptors are uniformly distributed on smooth muscle cells of human distal bronchi (Mak & Barnes, 1990). A dense nerve supply has been reported throughout the smooth muscle in human airways (Partanen *et al.*, 1982; Daniel *et al.*, 1986). These reports and our physiological results suggest that cholinergic nerve endings are co-localized with AChE, but not with BChE. Excess ACh (present in tissues treated with neostigmine) may lead to a diffusion of this neurotransmitter from the parasympathetic varicosities to the smooth muscle regions where hydrolysis may occur via BChE. This may explain the increased sensitivity to neostigmine which was observed in human airways in the presence of iso-OMPA. These data suggest that neurotransmitter hydrolysis may be controlled by a dual enzymatic process: the primary regulatory, namely AChE, responsible for the local neuronal synaptic regulation of ACh and BChE a secondary system, to protect against an increased release of ACh or against extra-neuronal appearance of ACh.

The results derived from human bronchial preparations, confirm previously published data in animal tissues concerning the different substrates for cholinesterases (Mittag *et al.*, 1971; Adler & Filbert, 1990). ACh is degraded by both AChE and BChE while BuCh is principally hydrolysed by BChE (Table 2). In some protocols the supernatant samples obtained following cholinesterase inhibitor treatments contained slightly higher quantities of the exogenously added substrates (0.5 μmol ACh or BuCh). This may have been due to protection of the endogenous production of ACh in presence of cholinesterases inhibitors.

Kirkpatrick & Rooney (1982) have suggested that in bovine tracheal muscle, neostigmine may act not only by preventing endogenous ACh degradation but also by stimulating release of endogenous ACh from nerve terminals. This notion was supported by the potentiation of maximal contractions to histamine, carbachol and acetylcholine in the bovine tracheal muscle. However, the sensitivity to these agents were unaffected. In contrast, the results presented here demonstrate that a higher concentration of neostigmine shifted the ACh concentration-effect curves to the left, while the methacholine curves were unaltered.

Iso-OMPA (10 μM) was a selective inhibitor of BChE. However, a higher concentration of iso-OMPA (100 μM) appeared not to be selective, since subsequent to 3 h of incubation, the protection against ACh enzymatic degradation was maximal (see Table 2). However, treatment of tissues for a shorter period (30 min or 1 h) with iso-OMPA (100 μM) selectively inhibited BChE, and these results from human airways were similar to those previously described (Traina & Serpietri, 1984; Thomsen *et al.*, 1991).

The data derived from the physiological studies (contractions) as well as results obtained in the biochemical protocols suggest that the BChE activity detected in human bronchi is associated with the enzymatic degradation of acetylcholine in these tissues.

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