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Involvement of K^+ channels in the relaxant effect of vasoactive intestinal peptide and atrial natriuretic peptide in isolated guinea-pig trachea

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Abstract

The possible contribution of K^+ channel activation to airway smooth muscle relaxation induced by vasoactive intestinal peptide (VIP) and atrial natriuretic peptide (ANP) was investigated in isolated guinea-pig trachea. Concentration-relaxation (CR) curves were assessed in preparations precontracted by 30 mM K^+, 124 mM K^+ or histamine either alone or in the presence of a K^+ channel blocker: iberiotoxin (IbTX), glipizide, tetraethylammonium (TEA) or Ba^{2+}. VIP completely relaxed contractions induced by histamine but had a lower effectiveness against those induced by 30 mM K^+ and 124 mM K^+. IbTX and TEA shifted the CR curve for VIP 5 and 14 times to the right, respectively. Glipizide and Ba^{2+} did not significantly antagonize the action of VIP. ANP relaxed contractions induced by histamine and 30 mM K^+ but failed to relax those elicited by 124 mM K^+. IbTX and TEA shifted the CR curve for ANP 8 and 46 times to the right, respectively. Glipizide and Ba^{2+} suppressed the maximal effect produced by ANP, and glipizide also shifted the CR curve to the left. The K^+ channel opener levcromakalim relaxed tracheal contractions induced by histamine and 30 mM K^+ but not those induced by 124 mM K^+. Glipizide caused a 5-fold rightward shift of the CR curve for levcromakalim whereas IbTX shifted the curve to the left and increased the maximal relaxant effect. The Ca^{2+} channel blocker isradipine completely relaxed contractions induced by 30 mM K^+ and 124 mM K^+ but only partially relaxed those contracted by histamine. All four K^+ channel blockers increased the maximal relaxant effect and shifted the CR curve for isradipine to the left. The results suggest that airway smooth muscle relaxation produced by VIP and ANP involves activation of large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) and further that ANP may possibly activate other types of K^+ channels additional to BK_{Ca}.

Keywords: ANP (atrial natriuretic polypeptide); VIP (vasoactive intestinal polypeptide); K^+ channel; Levcromakalim; Ca^{2+} channel blocker; Isradipine; Trachea; Smooth muscle, airway

1. Introduction

Several neuropeptides including vasoactive intestinal peptide (VIP) and atrial natriuretic peptide (ANP) have attracted interest in airway pharmacology. VIP has been shown to produce bronchodilation and to protect against histamine-induced bronchospasm in asthmatic patients (Morice et al., 1983). VIP produces airway smooth muscle relaxation in vitro (Hand et al., 1984) and together with nitric oxide (NO) it has been shown to be a mediator of inhibitory non-adrenergic non-cholinergic neurotransmission (i-NANC) in guinea-pig airways (Matsuzaki et al., 1980; Li and Rand, 1991). In human airways the role of VIP as a mediator of i-NANC has been questioned (Belvisi et al., 1992), but it presumably plays a significant role in regulation of airway tone through modulation of cholinergic neurotransmission (Aizawa et al., 1994). VIP is believed to act at specific receptors to stimulate adenylate cyclase, thereby causing an increased intracellular level of cAMP (Frandsen et al., 1978). However, whereas inhibition of phosphodiesterase in guinea-pig trachea potentiated the relaxant effect of two cAMP forming drugs, isoprenaline (a β_2-adrenoceptor agonist) and forskolin (a adenylate cyclase stimulator), it failed to enhance that of VIP, suggesting that mechanisms other than elevation of cAMP might be involved (Shikada et al., 1991).

Like VIP, ANP has been investigated as a bronchodila-

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tor in vivo. In asthmatic subjects inhalation or infusion of ANP causes bronchodilation (Hulks and Thomson, 1994) and protects against bronchoconstriction induced by inhalation of either methacholine or histamine (Angus et al., 1994). Some of the effects of ANP are mediated through binding at specific receptors and subsequent activation of the particulate form of guanylate cyclase, resulting in elevation of intracellular c-GMP (Anand Srivastava and Trachte, 1993). In guinea-pig trachea in vitro the relaxant effect of ANP is attenuated in preparations precontracted by 40 mM K^+ (Watanabe et al., 1990). Such low effectiveness against contraction induced by highly elevated extracellular K^+ might suggest that part of the relaxation could be mediated by opening of K^+ channels.

The aim of the present study was to investigate the possible involvement of K^+ channels in the relaxant actions of VIP and ANP in isolated guinea-pig trachea. Levcromakalim was investigated as a reference opener of ATP-sensitive K^+ channels and the dihydropyridine-type Ca^{2+} channel blocker isradipine as a compound producing smooth muscle relaxation by a mechanism different from K^+ channel opening.

2. Materials and methods

2.1. Tracheal preparations and measurement of contractile force

Dunkin-Hartley guinea-pigs (321 ± 12 g; $n = 25$) were killed by cervical dislocation. The trachea was removed and placed in cold oxygenated Krebs solution. It was carefully cleaned of adhering tissue under a dissecting microscope and cut into tubular segments comprising two adjoining cartilage rings (length approximately 2 mm). Six tracheal rings were each transferred to a 5-ml tissue bath containing Krebs solution (37°C; pH 7.4) and were mounted in precision myographs (Nielsen-Kudsk et al., 1986a) for measurement of isometric force. Six experiments were run in parallel. Each ring was suspended at a passive force of 0.6 g, which was optimal for development of contractile force in response to histamine and K^+ depolarisation. The amplified transducer signals were recorded on a six-channel recorder (Graphtec WR3101, Japan). The cyclooxygenase inhibitor indomethacin (2 μM) was present throughout the experiments in order to prevent spontaneous tone and assure reproducible responses to K^+ depolarisation (Nielsen-Kudsk et al., 1986b). The preparations equilibrated for 60 min before the start of experiments.

2.2. Experiments

The relaxant effect of VIP (1 nM to 1 μM), ANP (1 nM to 1 μM), levcromakalim (1 nM to 30 μM) and isradipine (0.1 nM to 0.3 μM) were investigated in airway smooth muscle preparations contracted by histamine (1 μM), K^+ (30 mM) or K^+ (124 mM). In histamine-contracted tracheas, the relaxant effects were evaluated both in the absence and in the presence of a K^+ channel blocker. The K^+ channel blockers used were: iberiotoxin (IbTX; 0.1 μM), tetraethylammonium (TEA; 8 mM), glipizide (10 μM) and Ba^{2+} (100 μM). Iberiotoxin, a scorpion venom, is a highly selective blocker of large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) (Garcia et al., 1991). Antidiabetic sulphonylureas, such as glibenclamide and glipizide, are selective blockers of ATP-sensitive K^+ channels (K_{ATP}). These agents possess the additional ability to relax tracheal smooth muscle (Nielsen-Kudsk and Thirstrup, 1993). Glipizide is less effective as a tracheal relaxant than glibenclamide but is still a potent blocker of K_{ATP} (Nielsen-Kudsk and Thirstrup, 1993). TEA concentrations in the range 0.1–10 mM preferentially block BK_{Ca} but other K^+ channels are inhibited at concentrations higher than 1 mM (Small et al., 1993). Ba^{2+} is an unselective K^+ channel blocker.

VIP, ANP, levcromakalim or isradipine were added cumulatively at intervals of about 10 min. This allowed stable contraction levels to develop between concentration increments. K^+ channel blockers were added to the histamine-precontracted preparations 25 min before addition of relaxant compounds. Precontraction by K^+ depolarization was produced by exchanging the tissue-bath solution with an isoosmolar K^+ Krebs solution (30 or 124 mM K^+). Experiments involving isradipine were carried out in dim light to avoid photodegradation of the compound.

2.3. Data analysis and statistics

All data are expressed as means ± S.E.M. Relaxant effects are expressed as the percentual reduction in contractile force relative to the precontraction level. Return to baseline was taken as 100% relaxation. Concentration-effect curves were made by fitting the mean data by non-linear regresson analysis to the Hill function $E = E_{max}/(1 + (10^{\log\ EC50}/10^{\log\ C})^S)$. GraphPad Prism, version 1.03 (GraphPad Software, USA) was used for the computer-fitting procedure. E_{max} is the theoretical maximal effect, EC_{50} is the concentration at which $E = 0.5E_{max}$ and S is the Hill coefficient related to the slope of the curve. The negative logarithm to EC_{50} is termed pEC_{50} (Jenkinson et al., 1995). Statistical evaluations were performed using the two-tailed *t*-test for paired and unpaired data with a significance level of 5%.

2.4. Drugs and solutions

The following compounds were used: histamine dihydrochloride, indomethacin, iberiotoxin (IbTX), tetraethylammonium (TEA) (Sigma, USA), glipizide (gift from Pfizer, USA), $BaCl_2$ (Merck, Germany), levcromakalim (gift from SmithKline Beecham, UK), isradipine (Sandoz, Schwitzerland), atrial natriuretic peptide and vasoactive intestinal peptide (gifts from NOVO-Nordisk, Denmark).

Histamine (10 mM), TEA (0.1 M), IbTX (10 μM) and $BaCl_2$ (10 mM) were dissolved in distilled water. Indomethacin (8.38 mM) was dissolved in 5% $NaHCO_3$. Glipizide (10 mM) was dissolved in a mixture of 1 ml NaOH (0.1 M) and 4 ml glucose (50 g/l). Levcromakalim (10 mM) was dissolved in 70% ethanol. Isradipine (1 mM) was dissolved in 96% ethanol. ANP (0.1 mM) and VIP (0.1 mM) were dissolved in 0.05 M acetic acid.

The composition of the Krebs solution was (in mM): NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15 and glucose 5.5. The K^+ rich Krebs solutions (30 mM or 124 mM K^+) were made by replacing equimolar amounts of NaCl with KCl to maintain isoosmolarity. The Krebs solutions were continuously gassed with a mixture of 95% O_2 and 5% CO_2.

3. Results

3.1. Contractile agents and K^+ channel blockers

Histamine (1 μM), 30 mM K^+ or 124 mM K^+ all produced contractions of similar magnitude. Histamine and 30 mM K^+ each produced a contractile response which was monophasic and sustained (2.40 ± 0.17 g, $n = 25$ and 2.40 ± 0.14 g, $n = 21$, respectively), whereas the response to 124 mM K^+ Krebs was biphasic with an initial small rapid phasic contraction followed by a sustained tonic contraction (2.39 ± 0.15 g, $n = 23$).

IbTX (0.1 μM) caused a slowly developing additional increase in histamine-induced tone, which stabilized at a significantly higher level after 20–25 min (2.62 ± 0.20 g vs. 3.65 ± 0.23 g; $n = 20$; $P < 0.0001$). TEA (8 mM) produced an initial rapid contractile response followed by a sustained elevation of tone (1.95 ± 0.16 g vs. 2.68 ± 0.22; $n = 24$; $P < 0.0001$). Glipizide (10 μM) did not influence histamine-induced tone (2.47 ± 0.20 g vs. 2.41 ± 0.22 g; $n = 23$; $P = 0.08$), whereas Ba^{2+} produced a slight but statistically significant elevation of tone (2.40 ± 0.22 g vs. 2.55 ± 0.22 g; $n = 24$; $P = 0.003$). Representative tracings demonstrating the effect of each of the four potassium channel blockers on histamine induced tone are shown in Fig. 1.

3.2. VIP

VIP produced concentration-dependent and complete relaxation of tracheal preparations precontracted by histamine. The presence of IbTX or TEA resulted in a significant rightward shift of the concentration-effect curve for VIP without any change in efficacy. TEA was the most potent, producing a 14× rightward shift of the concentration-effect curve, whereas IbTX shifted the curve 5× to the right. The relaxant action of VIP was not influenced by pretreatment with Ba^{2+} whereas the sulfonylurea glipizide produced a slight but significant leftward displacement of the concentration-effect curve. The derived pharmacodynamic data are stated in Table 1 and the concentration-effect curves are shown in Fig. 2.

VIP produced concentration-dependent but incomplete relaxation of both 30 mM and 124 mM K^+-induced contractions (Fig. 3). Calculated pEC_{50} and E_{max} values were not significantly different in preparations contracted with 30 mM K^+ compared with 124 mM K^+ (pEC_{50}: 7.36 ± 0.03 vs. 7.17 ± 0.23; $n = 5$ ($P = 0.44$) and E_{max}: 73.9 ± 1.71% vs. 69.7 ± 9.45 %; $n = 5$ ($P = 0.67$), respectively) but the corresponding S values were significantly different (1.16 ± 0.08 vs. 0.70 ± 0.14; $n = 5$ ($P = 0.02$)).

3.3. ANP

ANP caused concentration-dependent relaxation of tracheal rings precontracted by histamine. At the highest

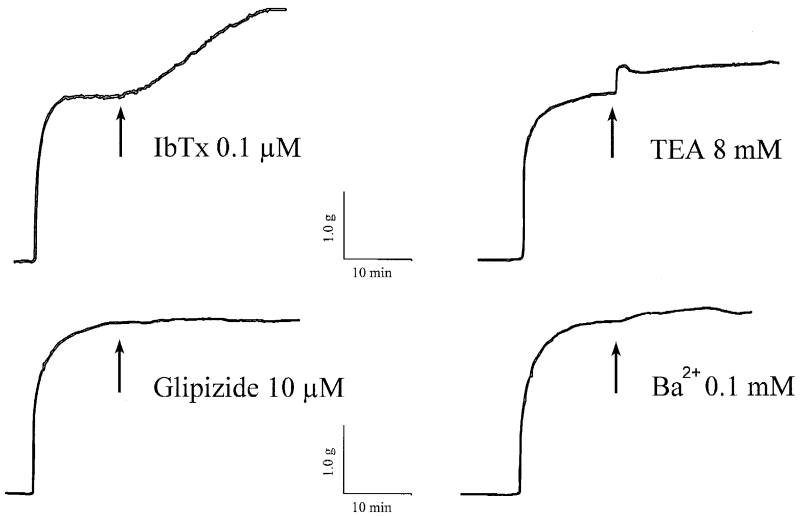

Fig. 1. Tracing showing the effects of four different K^+ channel blockers on established tracheal tone induced by histamine (1 μM). The compounds were added as indicated by the arrows. IbTX = iberiotoxin; TEA = tetraethylammonium.

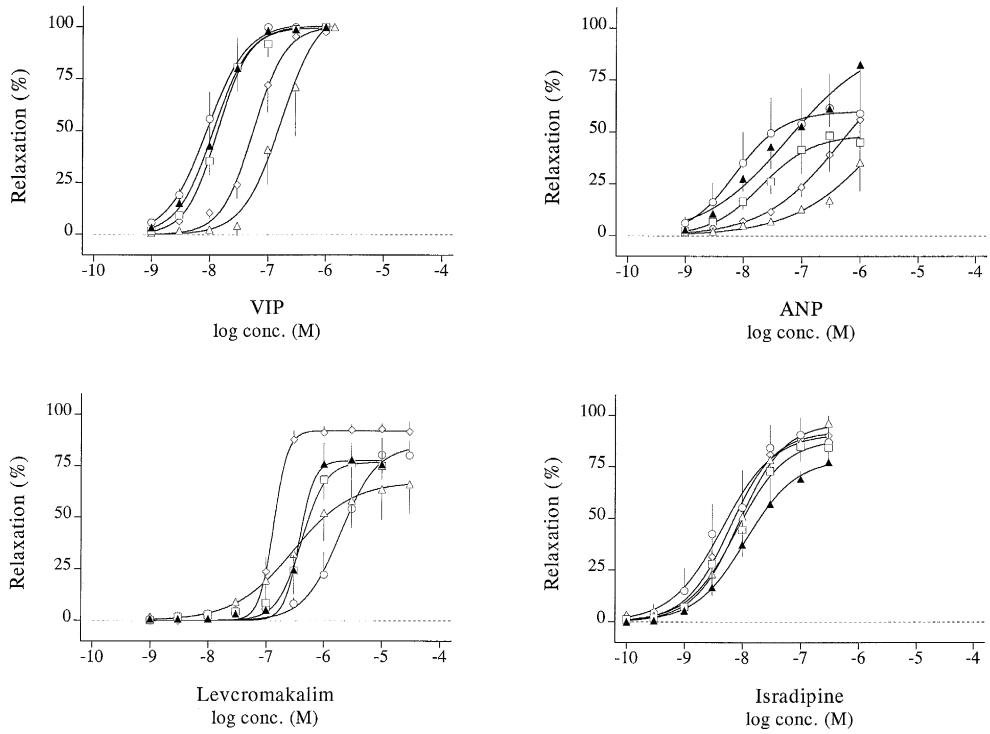

Fig. 2. Log concentration-relaxation curves for VIP, ANP, levcromakalim and isradipine in isolated guinea-pig trachea. Tracheal tone was induced by histamine (1 μM) and the compounds were added either in the absence (▲) or in the presence of one of the K^+ channel blockers: IbTX 0.1 μM (◇), TEA 8 mM (△), glipizide 10 μM (○) or Ba^{2+} 0.1 mM (□). S.E.M. values are shown as vertical bars ($n = 4–10$).

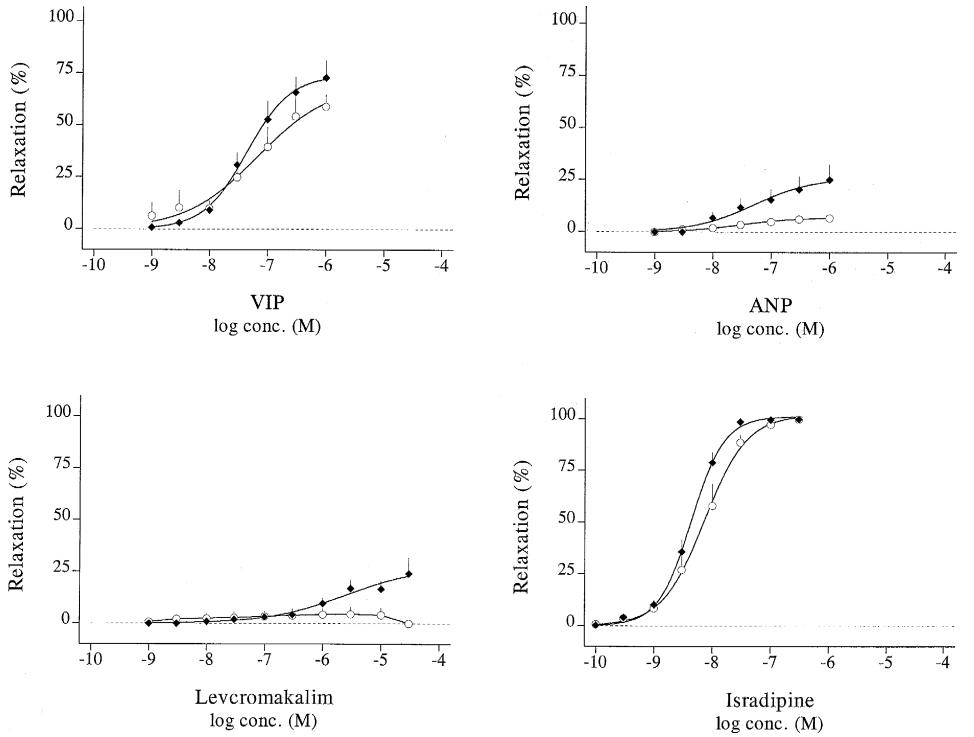

Fig. 3. Log concentration-relaxation curves for VIP, ANP, levcromakalim and isradipine in isolated guinea-pig trachea. Tracheal tone was induced either by 30 mM K^+ (♦) or 124 mM K^+ (○). S.E.M. values are shown as vertical bars ($n = 5–7$).

Table 1
Pharmacodynamic parameters (pEC_{50} ($=-\log(EC_{50})$), E_{max} and S) and S.E.M. obtained by iterative, non-linear regression analysis of mean concentration-relaxation data for VIP, ANP, levcromakalim and isradipine in isolated guinea-pig trachea precontracted by histamine (1 μM)

	pEC_{50}	E_{max}	S	EC_{50} ratio	E_{max} ratio
VIP					
Control	7.93 ± 0.02	100 ± 1	1.40 ± 0.09		
IbTX	7.25 ± 0.05 [a]	100 ± 4	1.58 ± 0.22	4.8	1.0
TEA	6.77 ± 0.07 [a]	109 ± 7	1.33 ± 0.22	14	1.1
Glipizide	8.04 ± 0.03 [a]	101 ± 2	1.26 ± 0.11	0.8	1.0
Ba^{2+}	7.86 ± 0.03	99.6 ± 1.1	1.60 ± 0.15	1.2	1.0
ANP					
Control	7.22 ± 0.33	94.1 ± 15.6	0.60 ± 0.15		
IbTX	6.33 ± 0.12 [a]	90.3 ± 7.8	0.66 ± 0.04	7.8	1.0
TEA	5.56 ± 0.09 [a]	94 (fixed)	0.58 ± 0.06	46	1.0
Glipizide	8.13 ± 0.04 [a]	60.1 ± 1.4	1.07 ± 0.10 [a]	0.1	0.6
Ba^{2+}	7.68 ± 0.09	48.6 ± 2.4 [a]	0.99 ± 0.15	0.3	0.5
Levcromakalim					
Control	6.43 ± 0.03	77.4 ± 1.8	3.32 ± 0.84		
IbTX	6.87 ± 0.02 [a]	92.0 ± 0.9 [a]	3.57 ± 0.43	0.4	1.2
TEA	6.53 ± 0.03	67.2 ± 0.6 [a]	0.86 ± 0.05 [a]	0.8	0.9
Glipizide	5.71 ± 0.06 [a]	84.7 ± 4.0	1.39 ± 0.21 [a]	5.2	1.1
Ba^{2+}	6.39 ± 0.04	76.7 ± 2.7	2.06 ± 0.35	1.1	1.0
Isradipine					
Control	7.94 ± 0.03	78.8 ± 1.6	1.01 ± 0.06		
IbTX	8.10 ± 0.07	88.5 ± 3.5 [a]	1.04 ± 0.13	0.7	1.1
TEA	8.06 ± 0.04	96.3 ± 2.5 [a]	1.11 ± 0.10	0.8	1.2
Glipizide	8.34 ± 0.09 [a]	91.3 ± 4.5 [a]	0.98 ± 0.17	0.4	1.2
Ba^{2+}	8.21 ± 0.04 [a]	92.1 ± 2.3 [a]	1.13 ± 0.11	0.5	1.2

The effects of the drugs were evaluated in the absence (control) or presence of either iberiotoxin (IbTX 0.1 μM), tetraethylammonium (TEA 8 mM), glipizide (10 μM) or Ba^{2+} (0.1 mM). The relative displacement of the concentration-effect curves produced by K^+ channel blockade is expressed as the EC_{50} ratio = EC_{50}(K^+ channel blocker present)/EC_{50}(control). The relative suppression of maximal effects produced by K^+ channel blockade is expressed as the E_{max} ratio = E_{max}(K^+ channel blocker present)/E_{max}(control). [a] $P < 0.05$ compared to control ($n = 4$–10).

concentration used (1 μM) it produced 82.4 ± 8.6% relaxation. The computer-derived pEC_{50} and E_{max} were 7.22 ± 0.33 and 94.1 ± 15.6% ($n = 6$), respectively. The relaxant profile was significantly influenced by each of the four K^+ channel blockers tested. Pretreatment with IbTX shifted the concentration-effect curve 8× to the right. In the presence of TEA the relaxant response to the highest concentration of ANP (1 μM) was 35.4 ± 13.9% (non-linear regression analysis did not convege). Setting E_{max} equal to E_{max} for the control tissue, a pEC_{50} was calculated to be 5.56 ± 0.09 ($n = 5$), equivalent to a 46× rightward shift of the curve. Pretreatment with Ba^{2+} or glipizide suppressed the maximal relaxant effect of ANP and glipizide also shifted the concentration-effect curve to the left (cf., Table 1 and Fig. 2).

ANP had a low relaxant activity against contractions induced by K^+ depolarization with a preferential relaxant effect on tissues contracted by 30 mM K^+ (pEC_{50}: 7.30 ± 0.21; E_{max}: 26.03 ± 3.37; S: 0.85 ± 0.22; $n = 6$) compared with 124 mM K^+ (E_{max}: 6.7 ± 1.6%; $n = 6$) (see Fig. 3).

3.4. Levcromakalim

In preparations precontracted by histamine, levcromakalim produced about 80% relaxation. IbTX produced a leftward displacement of the concentration-effect curve and an elevation of the E_{max} for levcromakalim. Both alterations were statistically significant. Pretreatment with TEA significantly reduced the maximal relaxation induced by levcromakalim to about 67% without any influence on potency. In contrast, glipizide caused a 5× rightward displacement of the concentration-effect curve for levcromakalim without any change in E_{max}. Incubation with Ba^{2+} was without significant effect on the relaxant action of levcromakalim.

Levcromakalim failed to relax tracheal preparations contracted by 124 mM K^+ whereas contractions induced by 30 mM K^+ were relaxed with a E_{max} of 27.17 ± 4.56% (pEC_{50}: 5.61 ± 0.30; S: 0.66 ± 0.14; $n = 5$). Concentration-effect curves for levcromakalim are shown in Fig. 2 and Fig. 3 and derived pharmacodynamic data are given in Table 1.

3.5. Isradipine

Isradipine produced about 80% relaxation in preparations precontracted by histamine. Pretreatment with IbTX resulted in an elevation of E_{max} and a leftward displacement of the concentration-effect curve for isradipine. Both alterations were statistically significant. TEA and Ba^{2+} each produced a significant elevation of E_{max} and each caused a non-significant leftward displacement of the concentration-effect curve for isradipine. Glipizide caused an elevation of E_{max} for isradipine and shifted the concentration-effect curve to the left. Concentration-effect curves for isradipine are shown in Fig. 2 and derived pharmacodynamic data are given in Table 1.

Isradipine produced concentration-dependent and complete relaxation of both 30 mM and 124 mM K^+-induced contractions (E_{max}: 101 ± 1 vs. 102 ± 2; $n = 5$ ($P = 0.69$)) with a slight preference for contractions induced by 30 mM K^+ (Fig. 3) (pEC_{50}: 8.36 ± 0.02 vs. 8.13 ± 0.03; $n = 5$ ($P < 0.0001$) and S: 1.54 ± 0.08 vs. 1.19 ± 0.03; $n = 5$ ($P = 0.01$), respectively).

4. Discussion

Agents which open K^+ channels preferentially relax airway smooth muscle contracted by moderately raised extracellular K^+ (< 40 mM) as compared with highly elevated concentrations of K^+ (Allen et al., 1986;

Nielsen-Kudsk and Thirstrup, 1993). VIP showed about the same relaxant profile against 30 mM K^+ and 124 mM K^+ without there being significant differences in pEC_{50} and E_{max} values. However, there was a tendency for selectivity against 30 mM K^+, resulting in a significantly steeper concentration-effect curve. VIP was less potent and showed reduced E_{max} in K^+-contracted preparations compared to histamine-contracted tissues. Both TEA and IbTX antagonized the tracheal relaxation induced by VIP in a competitive manner, suggesting that VIP activates BK_{Ca}. The additional contraction produced by the BK_{Ca} blockers iberiotoxin and TEA when added to histamine-precontracted preparations indicates that BK_{Ca} channels are open during airway smooth muscle contraction and counterregulate the agonist-induced level of tone. This further elevation of histamine-induced tone caused by addition of IbTX and TEA could theoretically contribute to the inhibition of VIP- and ANP-induced relaxation. However, this seems unlikely because the K^+ channel blockers failed to antagonize the relaxant action of isradipine, a drug acting by a mechanism of action different from K^+ channel opening. Moreover, IbTX and TEA had a more pronounced inhibitory effect against ANP than VIP, and IbTX potentiated rather than antagonized the relaxation induced by levcromakalim. VIP could possibly open BK_{Ca} directly, but another mechanism could be activation of protein kinase A and G due to stimulation of adenylate cyclase and subsequent elevation of cAMP. Activation of protein kinases A and G has been reported to result in phosphorylation and activation of BK_{Ca} (Kume et al., 1994; Torphy, 1994). Such a cAMP-dependent mechanism is supported by the finding that the effects of various cAMP-forming drugs (isoprenaline, salbutamol, theophylline) and drugs mimicking cAMP (dibutyryl cAMP) are antagonized by charybdotoxin and IbTX (Jones et al., 1990, 1993). Glipizide was without antagonistic effect on the tracheal relaxation induced by VIP, indicating that opening of K_{ATP} is not involved during this process. In isolated cerebral arteries VIP has been reported to act as an endogenous opener of K_{ATP} (Standen et al., 1989). The slight, but significant, leftward displacement of the concentration-effect curve for VIP produced by glipizide might possibly be due to the previously described relaxant properties of this drug (Nielsen-Kudsk and Thirstrup, 1993).

The difference in the relaxant response to ANP against 30 mM K^+ and 124 mM K^+ indicates that K^+ channel opening might be involved in the mechanism of action of ANP. The relaxation produced by ANP in histamine-contracted trachea was significantly inhibited by both IbTX and TEA, suggesting that opening of BK_{Ca} might also be involved. ANP has previously been shown to stimulate the particulate form of guanylate cyclase which causes elevation of intracellular c-GMP (Watanabe et al., 1990). The putative activation of BK_{Ca} by ANP might possibly result from elevation of intracellular c-GMP. An ANP-induced increase in c-GMP-sensitive outward K^+ current has been demonstrated by patch-clamp recordings in isolated rabbit aortic smooth muscle cells (Bkaily, 1990) and inhibitors of BK_{Ca} (IbTX, charybdotoxin and TEA) have been shown to antagonize relaxation produced by activators of the soluble form of guanylate cyclase (e.g. sodium nitroprusside and nitric oxide) in guinea-pig trachea (Bialecki and Stinson Fisher, 1995; Jones et al., 1990, 1993). The decrease of the maximal inhibitory effect of ANP in both glipizide- and Ba^{2+}-treated preparations suggests that other K^+ channels in addition to BK_{Ca} could be involved at high concentrations of ANP. ANP-induced activation of BK_{Ca}, K_{ATP} and other K^+ channels has been demonstrated in some non-airway tissues (Bkaily, 1990; White et al., 1993; Kubo et al., 1994) and it has recently been reported that relaxation of isolated bovine bronchi by urodilatin (a natriuretic peptide closely related to ANP) seems to involve opening of K_{ATP} and small conductance Ca^{2+}-activated K^+ channels (SK_{Ca}), but not opening of BK_{Ca} (Nally et al., 1995). Species differences and differences in structure between urodilatin and ANP could account for this discrepancy. The leftward shift of the concentration-relaxation curve for ANP, as for VIP, in the presence glipizide might be ascribed to the relaxant properties of this drug.

The results for levcromakalim are in accordance with earlier findings for K^+ channel openers in guinea-pig trachea (Nielsen-Kudsk and Thirstrup, 1993; Allen et al., 1986; Murray et al., 1991). Levcromakalim relaxed preparations contracted by 30 mM K^+ but not those contracted by 124 mM K^+. Glipizide antagonized the effect of levcromakalim in histamine-contracted preparations, indicating that levcromakalim is an opener of K_{ATP}. The leftward displacement of the levcromakalim concentration-relaxation curve by IbTX seems to indicate that blockade of BK_{Ca} potentiates the effect produced by K_{ATP} activation and is in agreement with previous studies on cromakalim in guinea-pig trachea (Jones et al., 1990, 1993). BK_{Ca} are activated during agonist-induced contractions (Kotlikoff, 1993) and the resulting hyperpolarizing effect could theoretically limit the hyperpolarizing action induced by pharmacological opening of K_{ATP}. Therefore, levcromakalim might possibly exert a greater hyperpolarization under conditions of BK_{Ca} blockade and this would potentiate its relaxant effect.

Isradipine completely relaxed contractions induced by both 30 mM K^+ and 124 mM K^+ and was less effective against histamine-induced contractions. Such a preferential relaxant effect against airway smooth muscle contraction induced by K^+ depolarization is a characteristic feature of Ca^{2+}-channel blockers (Nielsen-Kudsk et al., 1986b). It differed from the effect profile seen for VIP, ANP and levcromakalim. Addition of K^+ channel blockers to tracheal preparations with a sustained histamine-induced tone is expected to shift the membrane potential in a depolarizing direction and possibly make the contraction more dependent on the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels. Such a mechanism might possibly

explain the increased relaxant effectiveness of isradipine which was observed in the presence of each of the four K^+ channel blockers tested.

In conclusion, the results of this study suggest that airway smooth muscle relaxation by VIP and ANP involves opening of BK_{Ca}. ANP, but not VIP, might open K_{ATP} or other K^+ channels in addition to BK_{Ca}.

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