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Bradykinin increases Na^+-K^+ pump activity in cultured guinea-pig tracheal smooth muscle cells

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1 The effect of bradykinin on the Na^+-K^+ pump of airway smooth muscle was investigated by measuring ouabain-sensitive $^{86}Rb^+$ uptake in cultured guinea-pig tracheal smooth muscle cells.

2 Bradykinin induced a concentration-dependent increase in ouabain-sensitive $^{86}Rb^+$ uptake, with an EC_{50} of 3 nM ($pD_2 = 8.50 \pm 0.10$). Stimulation was not affected by indomethacin (1 μM) suggesting that it is not mediated by cycloxygenase products of arachidonic acid.

3 The B_1 receptor agonists Lys-des-Arg^9-bradykinin and des-Arg^9-bradykinin had no effect on ouabain-sensitive $^{86}Rb^+$ uptake. In contrast, the B_1 and B_2 receptor agonist Lys-bradykinin induced a concentration-dependent increase in ouabain-sensitive $^{86}Rb^+$ uptake with an EC_{50} of 6 nM ($pD_2 = 8.21 \pm 0.20$).

4 The B_1 receptor antagonist des-Arg^{10}-HOE 140 (1 μM) had no effect on bradykinin-stimulated ouabain-sensitive $^{86}Rb^+$ uptake. The B_2 receptor antagonists HOE 140 and WIN 64338 antagonized bradykinin-stimulated ouabain-sensitive $^{86}Rb^+$ uptake with pK_B values ($-\log$ M) of 8.20 ± 0.08 and 8.11 ± 0.20 respectively.

5 Reducing extracellular Na^+ from 146 mM to 11 mM caused a 53.5% decrease in basal ouabain-sensitive $^{86}Rb^+$ uptake and abolished bradykinin-induced uptake. Two inhibitors of the Na^+-H^+ exchanger, methylisobutyl-amiloride (MIA; 1–100 μM) and ethylisopropyl-amiloride (EIPA; 0.1–10 μM), inhibited bradykinin-stimulated ouabain-sensitive $^{86}Rb^+$ uptake without affecting basal uptake.

6 These results suggest that bradykinin increases Na^+-K^+ pump activity of guinea-pig tracheal smooth muscle *via* stimulation of B_2 receptors and activation of the Na^+-H^+ exchanger.
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Keywords: Airway smooth muscle; bradykinin; B_2 receptor; Na^+-H^+ exchanger; Na^+-K^+ pump; ouabain; rubidium-86; trachea

Abbreviations: BSS, balanced salt solution; EIPA, 5-(N-ethyl-N-isopropyl)-amiloride; MIA, 5-(N-methyl-N-isobutyl)-amiloride

Introduction

Bradykinin is an endogenous inflammatory peptide that is generated by plasma and tissue kallikreins from high and low molecular weight kininogens. A role for bradykinin in the pathogenesis of asthma has been suggested since increased levels of bradykinin are found in airways of asthmatic subjects (Christiansen *et al*., 1987) and inhalation of bradykinin by asthmatic subjects induces bronchoconstriction in part through cholinergic mechanisms (Fuller *et al*., 1987). *In vitro*, bradykinin induces contraction and/or relaxation of isolated airways through a number of direct and indirect mechanisms. Contraction results from activation of bradykinin receptors on airway smooth muscle (ASM), release of tachykinins from sensory nerve terminals and generation of thromboxane A_2 (Inoue *et al*., 1992; Hulsmann *et al*., 1994). On the other hand, bradykinin induces an epithelial-dependent relaxation of isolated airways through generation of inhibitory prostanoids and nitric oxide (Bramley *et al*., 1990; Schlemper & Calixto, 1994).

Bradykinin receptors are subdivided into two major subtypes, B_1 and B_2, which can be distinguished pharmacologically with selective agonists and antagonists (Regoli *et al*., 1993; 1994). Airway smooth muscle contraction is generally thought to be mediated by B_2 receptors (Trifilieff *et al*., 1992; Pruneau *et al*., 1995), although the existence of a novel B_3 receptor on guinea-pig tracheal smooth muscle has also been proposed (Farmer *et al*., 1989; Farmer & DeSiato, 1994). Bradykinin receptors on airway smooth muscle are coupled to activation of phospholipases A_2, C and D (Pyne & Pyne, 1993; Pyne *et al*., 1997), protein kinase C translocation (Pyne *et al*., 1994), phosphoinositide hydrolysis (Marsh & Hill, 1992), Ca^{2+} mobilization from intracellular and extracellular sources (Marsh & Hill, 1993), Ca^{2+} efflux (Farmer *et al*., 1991) and activation of mitogen-activated protein kinase (Pyne *et al*., 1997).

Airway smooth muscle possesses a Na^+-K^+ pump that contributes to the resting membrane potential and modulates tone. Activation of the pump with K^+ (in a K^+-free medium) results in hyperpolarization and relaxation of airway smooth muscle, whereas inhibition of the pump with ouabain causes depolarization and contraction (Souhrada *et al*., 1981; Chideckel *et al*., 1987; Gunst & Stropp, 1988). Na^+-K^+ pump activity is thought to alter smooth muscle tone as a result of changes in Ca^{2+} influx through voltage-dependent

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Ca^{2+} channels and Ca^{2+} efflux *via* the Na^+-Ca^{2+} exchanger. There is increasing evidence that the Na^+-K^+ pump is in turn influenced by bronchoactive mediators. Vasoactive intestinal peptide and 5-hydroxytryptamine both activate the Na^+-K^+ pump of airway smooth muscle as measured by increases in ouabain-sensitive $^{86}Rb^+$ uptake (Morrison & Vanhoutte, 1996; Rhoden *et al*., 2000).

The aim of the present study was to investigate the effect of bradykinin on the Na^+-K^+ pump of airway smooth muscle through measurements of ouabain-sensitive $^{86}Rb^+$ uptake in cultured guinea-pig tracheal smooth muscle cells. The results suggest that bradykinin stimulates the Na^+-K^+ pump *via* activation of B_2 receptors, and that stimulation is secondary to Na^+ influx through the Na^+-H^+-exchanger.

Methods

Cell culture

Cultured airway smooth muscle cells were derived from male Dunkin-Hartley guinea-pigs (Covance, Denver, PA, U.S.A.) weighing 250–500 g. Animals were euthanized with an overdose of sodium pentobarbitone (150 mg kg^{-1} i.p.) and the trachea harvested. The trachea was cut longitudinally through the cartilage and the epithelial layer was removed by rubbing the lumenal surface with a sterile cotton-wool probe. The trachealis muscle was dissected free from the cartilage, minced with scalpel blades into $\sim$1 mm^2 pieces and placed in culture dishes. Tissue explants were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% foetal bovine serum, 100 u ml^{-1} penicillin and 100 μg ml^{-1} streptomycin in humidified air containing 5% CO_2 at 37°C. The culture medium was changed 1 week after initial set up, and twice per week thereafter. Cells migrated from tissue explants and proliferated till confluence over a 2–3 week period. Cells were passaged with 0.05% trypsin and 0.53 mM EDTA followed by centrifugation at 1500 r.p.m. and resuspension in culture medium. Cells were maintained in a culture for up to five passages. For $^{86}Rb^+$ uptake studies, cells were plated onto 24-well plates at a density of $2-4\times10^4$ cells $well^{-1}$. Cells grown in Lab-Tek chamber slides were characterized as smooth muscle cells by immunohistochemistry using a labelled streptavidin-biotin-peroxidase kit (Zymed Laboratories, San Francisco, CA, U.S.A.). Positive staining was obtained with primary antibodies for smooth muscle-specific α-actin (Sigma, clone 1A4) and smooth muscle-specific myosin (Sigma, clone hSM-V).

Ouabain-sensitive $^{86}Rb^+$ uptake

Na^+-K^+ pump activity was measured in confluent tracheal smooth muscle cells as ouabain-sensitive $^{86}Rb^+$ uptake, as previously described (Rhoden *et al*., 2000). Uptake was measured in a balanced salt solution (BSS) containing (mM): NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10 and glucose 10 (pH 7.4) at 37°C. Cells were incubated for 5 min in BSS containing 10 μM bumetanide $\pm$ 100 μM ouabain, followed by 0.5–1 μCi ml^{-1} $^{86}Rb^+$ and bradykinin (10 pM–10 μM) for 10 min. Bumetanide was included in order to prevent uptake by the Na^+-K^+-$2Cl^-$ cotransporter and thereby reduce background. At the end of the uptake period, cells were washed six times in 1 ml ice-cold wash buffer. The wash buffer consisted of BSS containing RbCl instead of KCl in order to displace $^{86}Rb^+$ from extracellular sites. Cells were solubilized in Lowry reagent and total cellular protein measured by the Lowry method (Sigma protein assay kit P5656). Radioactivity was measured by liquid scintillation counting (3000–6000 DPM per sample counted for 10 min with a counting efficiency of 63%). Ouabain-sensitive uptake was calculated as the difference in uptake in the presence and absence of ouabain. The effect of extracellular Na^+ on Na^+-K^+ pump activity was investigated by replacing NaCl in BSS with equimolar choline chloride. Since the pH of BSS was corrected with NaOH, the final concentration of Na^+ in BSS was 146 mM (normal BSS) or 11 mM (low Na^+ BSS). Pretreatment with antagonists and inhibitors was carried out 20 min before the addition of $^{86}Rb^+$.

Drugs

Bradykinin, bumetanide, des-Arg^9-bradykinin, des-Arg^{10}-HOE 140, Lys-bradykinin, Lys-des-Arg^9-bradykinin, 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), 5-(N-methyl-N-isobutyl)-amiloride (MIA) and ouabain were purchased from Sigma (St. Louis, MO, U.S.A.). HOE 140 was purchased from Peninsula Laboratories (San Carlos, CA, U.S.A.) and WIN 64338 from Tocris (Ballwin, MO, U.S.A.). ^{86}RbCl was purchased from NEN Life Science Products (Boston, MA, U.S.A.).

Data analysis

Results are expressed as mean values $\pm$ s.e.mean of n experiments performed on duplicate or triplicate wells. Each n represents cells cultured from a different animal and/or passage. EC_{50} and pD_2 ($-\log EC_{50}$) values for agonists were determined by non-linear regression curve fitting of concentration-response data fitted to the equation $Y = Y_{min} + (Y_{max} - Y_{min})/(1 + [EC_{50}/X]^n)$, where Y_{min} and Y_{max} are the minimum and maximum responses respectively, X is the agonist concentration and n is the Hill slope (GraphPad Prism, San Diego, CA, U.S.A.). The pK_B value ($-\log$ M) for HOE 140 was derived from a Schild plot of $\log(DR-1)$ versus log[B] where DR is the agonist dose-ratio in the presence and absence of antagonist B. WIN 64338 appeared to cause insurmountable antagonism, and the pK_B was calculated from the equation $K_B = [B]/\text{slope} - 1$, the slope corresponding to the double reciprocal plot of equi-effective concentrations of agonist in the absence (1/A) and in the presence (1/A′) of antagonist B (Kenakin, 1984). Differences between groups were analysed by the Student's t-test or by ANOVA with the Bonferroni post-test for multiple comparisons (StatView, SAS Institute Inc., Cary, NC, U.S.A.). Statistical significance was assumed at a probability (P) value <0.05.

Results

Effect of bradykinin on $^{86}Rb^+$ uptake

Bradykinin (0.1 nM–10 μM) induced a concentration-dependent increase in $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells (Figure 1A). Ouabain decreased $^{86}Rb^+$ uptake by 86% and abolished the stimulation of

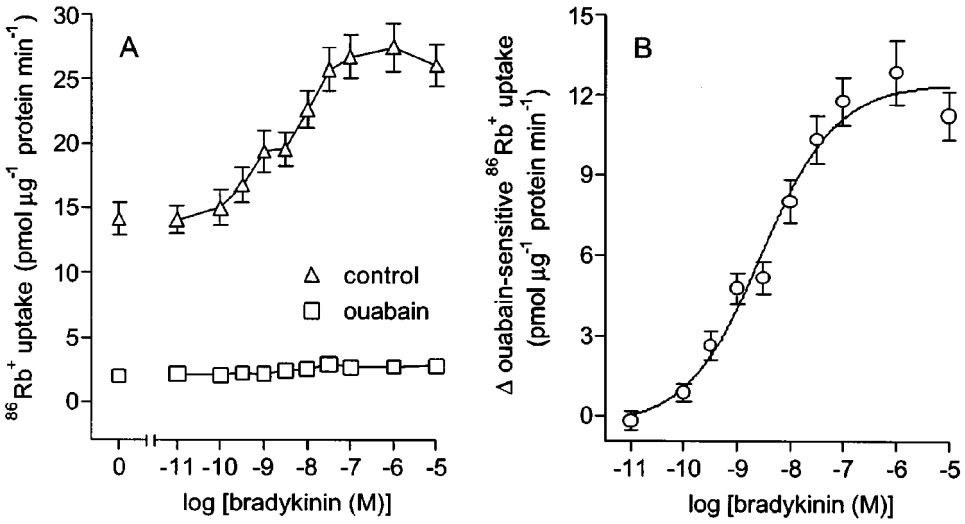

Figure 1 Effect of bradykinin on $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells. $^{86}Rb^+$ uptake is expressed as total uptake in the absence or presence of 100 μM ouabain (A) or as the increase in ouabain-sensitive uptake induced by bradykinin (B). Points represent mean ± s.e.mean ($n=17$).

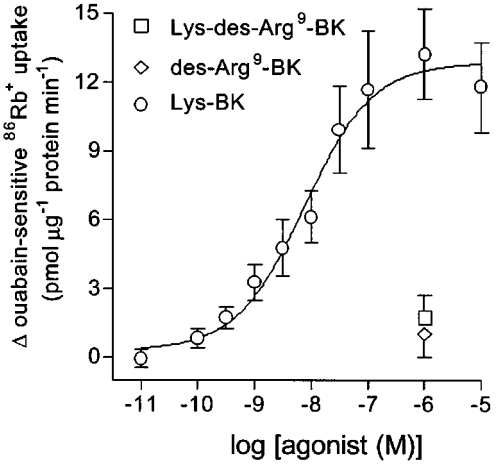

Figure 2 Effect of the bradykinin receptor agonists des-Arg^9-bradykinin, Lys-des-Arg^9-bradykinin and Lys-bradykinin on ouabain-sensitive $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells. Points represent mean ± s.e.mean ($n=6-8$).

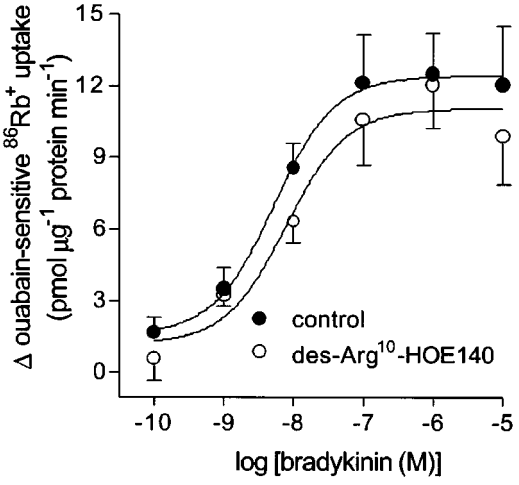

Figure 3 Effect of the bradykinin B_1 receptor antagonist des-Arg^{10}-HOE 140 (1 μM) on bradykinin-stimulated ouabain-sensitive $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells. Points represent mean ± s.e.mean ($n=8$).

uptake induced by bradykinin. Bradykinin increased ouabain-sensitive uptake with an EC_{50} of 3 nM ($pD_2=8.50\pm0.10$, $n=17$) (Figure 1B). At a maximally effective concentration of 1 μM, bradykinin increased ouabain-sensitive $^{86}Rb^+$ uptake from 12.05 ± 1.07 to 24.90 ± 1.46 pmol μg^{-1} protein min^{-1} suggesting a doubling of Na^+-K^+ pump activity. The ability of bradykinin to increase ouabain-sensitive $^{86}Rb^+$ uptake was not affected by 1 μM indomethacin, with similar pD_2 values in the absence (8.42 ± 0.14, $n=6$) and presence (8.44 ± 0.11, $n=6$) of indomethacin.

Receptor agonists and antagonists

The receptor mediating the bradykinin-induced increase in Na^+-K^+ pump activity was investigated with selective agonists and antagonists. The B_1 receptor agonists des-Arg^9-bradykinin (0.1 nM – 10 μM) and Lys-des-Arg^9-bradykinin (0.1 nM – 10 μM) had no effect on ouabain-sensitive $^{86}Rb^+$ uptake (Figure 2). In contrast, the B_1 and B_2 receptor agonist Lys-bradykinin induced a concentration-dependent increase in ouabain-sensitive $^{86}Rb^+$ uptake, with an EC_{50} of 6 nM ($pD_2=8.21\pm0.20$, $n=8$) (Figure 2). Maximal uptake induced by Lys-bradykinin was similar to that induced by bradykinin.

The B_1 receptor antagonist des-Arg^{10}-HOE 140 (1 μM) had no significant effect on bradykinin-induced ouabain-sensitive $^{86}Rb^+$ uptake (Figure 3), with pD_2 values for bradykinin of 8.65 ± 0.25 ($n=6$) in the absence and 8.35 ± 0.13 ($n=6$) in the presence of antagonist. The B_2 receptor antagonists HOE 140 and WIN 64338 inhibited bradykinin-induced ouabain-sensitive $^{86}Rb^+$ uptake (Figure 4). The Schild plot for HOE 140 yielded a straight line ($R=0.90$) with a slope (1.09 ± 0.10) that was not significantly different from unity. The pK_B value

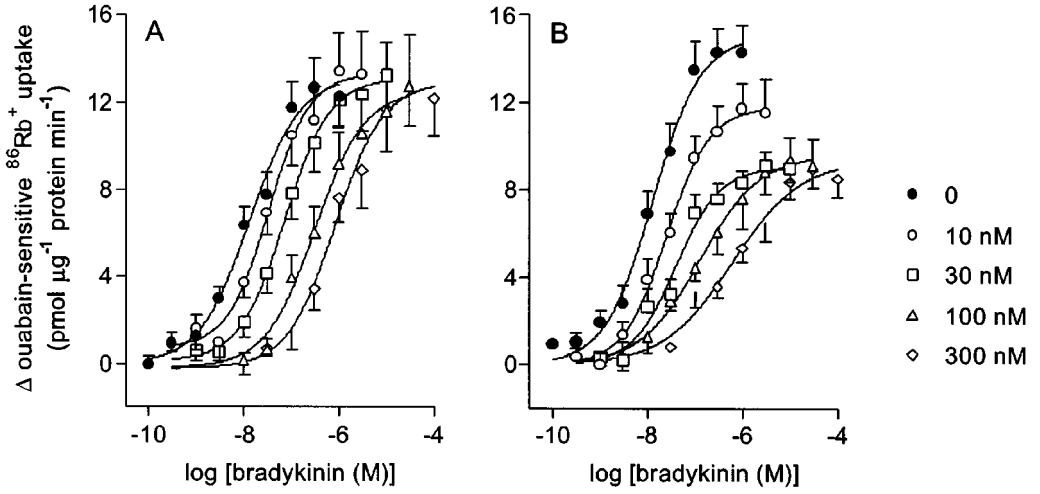

Figure 4 Effect of the bradykinin B_2 antagonists HOE 140 (A) and WIN 64338 (B) on bradykinin-stimulated ouabain-sensitive $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells. Uptake was measured in the presence of 0, 10, 30, 100 or 300 nM antagonist. Points represent mean ± s.e.mean ($n=8$ for HOE 140 and $n=9$ for WIN 64338).

(−log M) for HOE 140 derived from the Schild plot was 8.20 ± 0.08 ($n=8$). WIN 64338 caused a decrease in the maximal response to bradykinin suggesting insurmountable antagonism. The pK_B value (−log M) for WIN 64338, derived from the double-reciprocal plot of equi-effective concentrations of agonist in the presence and absence of antagonist, was 8.11 ± 0.20 ($n=9$). HOE 140 (10–300 nM) and WIN 64338 (10–300 nM) had no effect on resting ouabain-sensitive $^{86}Rb^+$ uptake in the absence of bradykinin.

Role of Na^+ influx and the Na^+-H^+ exchanger

The role of Na^+ influx in the stimulation of $^{86}Rb^+$ uptake by bradykinin was investigated by first examining the effect of Na^+ influx *per se* on ouabain-sensitive uptake. The Na^+ ionophore monensin (10 nM – 10 μM) induced a concentration-dependent increase in ouabain-sensitive $^{86}Rb^+$ uptake (Figure 5) confirming that an increase in Na^+ influx stimulates the Na^+-K^+ pump. In contrast, reducing Na^+ influx by decreasing the extracellular concentration of Na^+ from 146 mM to 11 mM caused a 53.5 ± 3.1% reduction in ouabain-sensitive $^{86}Rb^+$ uptake ($P<0.001$, $n=8$) (Figure 6). The effect of reducing the concentration of extracellular Na^+ was also investigated on the ability of bradykinin (1 μM) and an equi-effective concentration of monensin (0.3 μM) to increase Na^+-K^+ pump activity. In the presence of 11 mM Na^+, bradykinin and monensin both failed to induce a significant increase in ouabain-sensitive $^{86}Rb^+$ uptake (Figure 6), suggesting that Na^+ influx is necessary for bradykinin to increase Na^+-K^+ pump activity.

The role of the Na^+-H^+ exchanger in bradykinin-stimulated Na^+-K^+ pump activity was assessed with MIA and EIPA, two selective inhibitors of the Na^+-H^+ exchanger. MIA (1–100 μM) and EIPA 0.1–10 μM) both inhibited bradykinin-stimulated $^{86}Rb^+$ uptake in a concentration-dependent manner without affecting basal uptake in the absence of bradykinin (Figure 7).

Since amiloride analogues also inhibit voltage-dependent Ca^{2+} channels (Kleyman & Cragoe, 1988), the effect of nifedipine on the ability of bradykinin to stimulate ouabain-sensitive $^{86}Rb^+$ uptake was examined. Bradykinin (1 μM) increased ouabain-sensitive $^{86}Rb^+$ uptake by 13.3 ± 1.4 pmol μg^{-1} protein min^{-1} in the absence of nifedipine and 13.3 ± 1.8 pmol μg^{-1} protein min^{-1} ($n=6$) in the presence of 1 μM nifedipine, demonstrating that inhibition of L-type Ca^{2+} channels has no effect on the stimulation of the Na^+-K^+ pump activity by bradykinin.

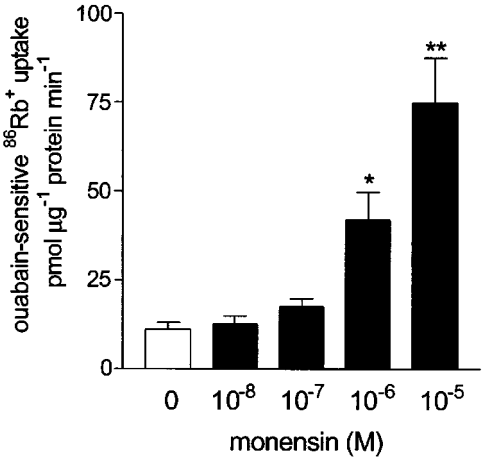

Figure 5 Effect of monensin on ouabain-sensitive $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells. $^*P<0.05$, $^{**}P<0.01$ vs no monensin, ANOVA with Bonferroni post-test. Points represent mean ± s.e.mean ($n=8-10$).

Discussion

In many cells, the Na^+-K^+ pump is regulated by hormones, neurotransmitters and growth factors. Regulation occurs as (i) acute changes in the activation kinetics of the pump due to changes in the concentration and/or affinity of substrates (usually Na^+), or (ii) through longer lasting changes in the density of active pumps on the plasma membrane due to translocation from intracellular stores or *de novo* synthesis

(Therien & Blostein, 2000). In airway smooth muscle, VIP and 5-hydroxytryptamine produce an acute increase in Na^+-K^+ pump activity, measured as ouabain-sensitive $^{86}Rb^+$ uptake, and this effect is mediated by an increase in Na^+ influx into cells (Morrison & Vanhoutte, 1996; Rhoden *et al*., 2000). The present study demonstrates that bradykinin also increases ouabain-sensitive $^{86}Rb^+$ uptake in cultured guinea-pig tracheal cells, and stimulation is comparable in magnitude to that produced in the same cell type by 5-hydroxytryptamine (Rhoden *et al*., 2000). Stimulation is also dependent upon extracellular Na^+ suggesting that it is secondary to an increase in Na^+ influx. The Na^+ ionophore monensin increased ouabain-sensitive $^{86}Rb^+$ uptake confirming that an increase in Na^+ influx *per se* can stimulate the Na^+-K^+ pump in airway smooth muscle.

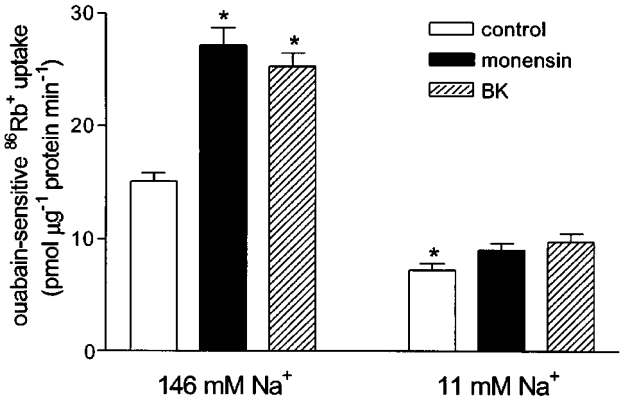

Figure 6 Effect of extracellular Na^+ concentration on the stimulation of ouabain-sensitive $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells by monensin (0.3 μM) and bradykinin (1 μM). Uptake was measured in BSS containing 146 mM Na^+ or 11 mM Na^+. $*P<0.05$ vs control in 146 mM Na^+, ANOVA with Bonferroni post-test. Points represent mean$\pm$s.e.mean ($n=8$).

Na^+ influx into cells occurs through Na^+ channels and through a number of Na^+-coupled transporters. The increase in Na^+-K^+ pump activity induced by bradykinin was inhibited by the amiloride analogues EIPA and MIA suggesting that it may be mediated by an increase in Na^+ influx *via* the Na^+-H^+ exchanger. The Na^+-H^+ exchanger exists in multiple isoforms that differ in their sensitivity to amiloride analogues (Yun *et al*., 1995). NHE1 is the ubiquitous amiloride-sensitive isoform that is inhibited by submicromolar EIPA and MIA (Kleyman & Cragoe, 1988). Other tissue-specific isoforms exhibit a 50–400 fold lower sensitivity to EIPA (Yun *et al*., 1995). The NHE isoform present in airway smooth muscle has not been determined, but the concentration-dependence for inhibition of bradykinin-induced $^{86}Rb^+$ uptake by EIPA and MIA may suggest the presence of alternate isoforms with a lower amiloride-sensitivity. High concentrations of EIPA and MIA (10–1000 μM) also inhibit other ion transport systems including the Na^+-K^+ pump, Na^+ channels, the Na^+-Ca^{2+} exchanger and voltage-dependent Ca^{2+} channels (Kleyman & Cragoe, 1988). EIPA and MIA had no effect on basal ouabain-sensitive $^{86}Rb^+$ uptake suggesting that these agents do not have a direct effect on the Na^+-K^+ pump in airway smooth muscle. Furthermore, resting Na^+-K^+ pump activity is maintained by Na^+ influx through amiloride-insensitive pathways. Voltage-dependent Ca^{2+} channels are unlikely to be involved in the effects of amiloride analogues since nifedipine, a more selective inhibitor of L-type Ca^{2+} channels, had no effect on bradykinin-stimulated $^{86}Rb^+$ uptake.

Bradykinin receptors can be classified pharmacologically into B_1 and B_2 subtypes according to the order of potency of agonists and antagonists (Regoli *et al*., 1993; 1994). The results of this study suggest that increase in Na^+-K^+ pump activity induced by bradykinin is mediated by B_2 receptors. Lys-des-Arg^9-bradykinin and des-Arg^9-bradykinin are B_1-selective agonists, and neither affected ouabain-sensitive $^{86}Rb^+$ uptake. In contrast, bradykinin and Lys-bradykinin stimulated $^{86}Rb^+$ uptake with similar pD_2 values of 8.50 and

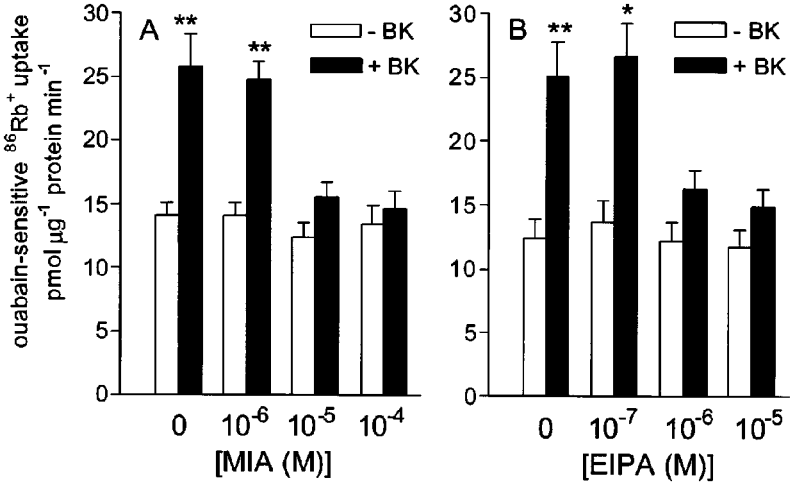

Figure 7 Effect of the Na^+-H^+ exchanger inhibitors MIA (A) and EIPA (B) on the stimulation of ouabain-sensitive $^{86}Rb^+$ uptake into cultured guinea-pig tracheal smooth muscle cells by bradykinin (1 μM). Uptake was measured in the presence of 1–100 μM MIA or 0.1–10 μM EIPA. $**P<0.001$, $*P<0.005$ vs no bradykinin, unpaired Student's *t*-test. Points represent mean$\pm$s.e.mean ($n=8$).

8.21 respectively, and this is in agreement with their potency on B_2 receptor systems (Regoli *et al*., 1993).

Stimulation of ouabain-sensitive $^{86}Rb^+$ uptake by bradykinin was antagonized by HOE 140 and WIN 64338 with pK_B values of 8.20 and 8.11 respectively. HOE 140 is a long-acting B_2 receptor antagonist with an apparent affinity (pA_2 value) of 8–9 in various bioassays (Hock *et al*., 1991; Regoli *et al*., 1993; 1994). HOE 140 displaces bradykinin binding from guinea-pig tracheal receptors and inhibits bradykinin-induced contraction of the guinea-pig trachea with a pK_B of 8.13 (Trifilieff *et al*., 1992; Pruneau *et al*., 1995). WIN 64338 is a nonpeptide B_2 receptor antagonist with pA_2 values of 7.1–8.2 on guinea-pig B_2 receptor systems (Regoli *et al*., 1994). WIN 64338 inhibited bradykinin-induced contraction of the guinea-pig trachea with pK_B values of 7.19 (Scherrer *et al*., 1995) and 7.36 (Pruneau *et al*., 1995). In contrast, Farmer *et al*. (1989) and Farmer & DeSiato (1994) failed to demonstrate any inhibitory effect of WIN 64338 and NPC 567, another B_2 antagonist, on bradykinin-induced contractions of the guinea-pig trachea leading the authors to propose the existence of a novel B_3 receptor in this preparation. Both HOE 140 and WIN 64338 have been shown to displace bradykinin binding from cultured guinea-pig tracheal smooth muscle cells confirming the presence of B_2 receptors on cultured as well as freshly-isolated smooth muscle (Scherrer *et al*., 1998). Cultured guinea-pig tracheal smooth cells are also reported to contain B_3 receptors (Farmer *et al*., 1991), but we have no evidence for their involvement in the regulation of the Na^+-K^+ pump by bradykinin.

WIN 64338 produced insurmountable antagonism of bradykinin-stimulated $^{86}Rb^+$ uptake. Although WIN 64338 acts as a competitive antagonist in most systems (Regoli *et al*., 1994), it causes insurmountable antagonism of guinea-pig tracheal contractions (Pruneau *et al*., 1995; Scherrer *et al*., 1995). Potential causes of insurmountable antagonism include (i) non-specific inhibition of post-receptor events; (ii) irreversible or noncompetitive antagonism; (iii) pseudo-irreversible or slowly-reversing antagonism due to slow dissociation of the antagonist from the receptor (Robertson *et al*., 1994); (iv) allosteric modulation of the receptor (Kaumann & Frenken, 1985); and (v) competitive antagonism against an indirect agonist (Kenakin, 1993).

HOE 140 acted as a competitive antagonist against bradykinin-stimulated $^{86}Rb^+$ uptake, with no change in the maximal response to bradykinin and a Schild plot slope of unity. In the guinea-pig isolated trachea, HOE 140 produces both surmountable and insurmountable antagonism of bradykinin-induced contractions (Pruneau *et al*., 1995; Trifilieff *et al*., 1992).

Both forms of antagonism also occur in other bioassays in a tissue and species-dependent manner (Rhaleb *et al*., 1992; Feletou *et al*., 1994), and have been confirmed through the controlled expression of recombinant B_2 receptors from different species (Bachvarov *et al*., 1995). In some systems, HOE 140 also acts as a partial agonist (Feletou *et al*., 1994) and an inverse agonist (Leeb-Lundberg *et al*., 1994). In our experiments, HOE 140 had no effect (stimulatory or inhibitory) on resting ouabain-sensitive $^{86}Rb^+$ uptake in the absence of bradykinin, suggesting that the last two properties of HOE 140 are not evident in this preparation.

The physiological relevance of bradykinin-induced stimulation of the Na^+-H^+ exchanger and the consequent increase in Na^+-K^+ pump activity is uncertain. Contraction of airway smooth muscle is associated with a transient decrease in intracellular pH (pH_i), followed by a slow recovery that it is mediated by the Na^+-H^+ exchanger (Bose *et al*., 1990). Thus, activation of the Na^+-H^+ exchanger by bradykinin may reflect the need to extrude excess protons produced during contraction in order to restore pH_i. An increase in Na^+ influx through the Na^+-H^+ exchanger would increase Na^+-K^+ pump activity in an attempt to restore resting [Na^+_i]. Since an increase in Na^+-K^+ pump activity is associated with hyperpolarization, this phenomenon may also represent a negative feedback mechanism that tends to oppose contraction in response to stimulation of B_2 receptors on airway smooth muscle. Such an autoregulatory mechanism has been proposed in other smooth muscles. Phorbol esters inhibit agonist-induced contractions of ileal smooth muscle through activation of the Na^+-K^+ pump, and cause a 24% increase in ouabain-sensitive $^{86}Rb^+$ uptake (Sasaguri & Watson, 1990). Similarly, serotonin attenuates contractions of isolated arteries by increasing Na^+-K^+ pump activity (Moreland *et al*., 1985), and causes a 287% increase in ouabain-sensitive $^{86}Rb^+$ uptake in cultured vascular smooth muscle cells (Navran *et al*., 1991). Activation of the Na^+-K^+ pump is also thought to mediate the after-hyperpolarization seen in ileal smooth muscle following acetylcholine application (Bolton, 1973). Clearly, measurements of membrane potential, [Na^+_i], pH_i and tone are needed to establish the physiological role of bradykinin-induced changes in ion transport observed in the present study.

In conclusion, bradykinin increases Na^+-K^+ pump activity of cultured airway smooth muscle *via* activation of B_2 receptors and stimulation of Na^+ influx through the Na^+-H^+ exchanger. Further studies are needed to determine the mechanism by which bradykinin activates the Na^+-H^+ exchanger and the functional implication for the regulation of airway smooth muscle tone by bradykinin.

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