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Corticosteroid modulation of Na^+-K^+ pump-mediated relaxation in maturing airway smooth muscle

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1 The ontogeny of the relaxant influence of the airway electrogenic Na^+-K^+ pump and its potential modulation by corticosteroids were examined in airway smooth muscle (ASM) segments isolated from newborn and adult rabbits.

2 Control and methylprednisolone-treated (MP) ASM segments were half-maximally contracted with methacholine in K^+-free buffer and the ASM relaxant responses to Na^+-K^+ pump activation were subsequently evaluated. Relative to adult ASM, control newborn ASM showed significantly enhanced maximal relaxation (R_{max}) to KCl ($62.5 \pm 5.2\%$ vs. $47.8 \pm 5.2\%$), but no difference in sensitivity ($pC_2 = -\log$ concentration producing 50% R_{max}: 2.18 ± 0.12 vs. $2.29 \pm 0.09 - \log$ M).

3 Exposure of ASM segments to 500 μM methylprednisolone for 1 h potentiated the airway Na^+-K^+ pump activity. A more pronounced effect was obtained in newborn ASM, where both the R_{max} and pC_2 values were significantly enhanced. In mature ASM, only the R_{max} response to KCl was increased in the presence of MP.

4 Collectively, these data demonstrate that: (i) the functional activity of the airway electrogenic Na^+-K^+ pump decreases with post-natal maturation in the rabbit: (ii) corticosteroid treatment potentiates Na^+-K^+ pump activity in rabbit ASM; and (iii) the latter effect of corticosteroids is enhanced in immature airways.

5 The above findings provide new evidence that the airway relaxant response to activation of the electrogenic Na^+-K^+ pump varies ontogenetically and that corticosteroids potentiate the Na^+-K^+ pump activity in an age-dependent manner.

Keywords: Sodium-potassium transporting adenosinetriphosphatase; methylprednisolone; maturation; trachea

Introduction

One of the principal factors regulating excitability of all contractile cells is the membrane potential (E_m), which is determined by the electrochemical distribution of ions across the cell membrane and the electrogenic component of the Na^+-K^+ pump. The presence of an electrogenic Na^+-K^+ pump in airway smooth muscle (ASM) was first identified by the observation that the resting E_m in tracheal smooth muscle cells diminished by 10 to 15 mV following pump inhibition with 10 μM ouabain or K^+-free buffer (Souhrada & Souhrada, 1981). This Na^+-K^+ pump contribution to the resting E_m in ASM is similar to the -10 to -20 mV measured in mature taenia coli (Matthews *et al.*, 1973) and vascular smooth muscle (Matthews & Sutter, 1967). Activity of the Na^+-K^+ pump is regulated by extracellular potassium ($[K^+]_e$), intracellular sodium ($[Na^+]_i$), and various hormones (Gick *et al.*, 1988). In particular, glucocorticoids have been shown to enhance the Na^+-K^+ pump activity in erythrocytes (Kaji *et al.*, 1981), nephron segments (Garg *et al.*, 1985), and colonic epithelium (Sellin & DeSoignie, 1985); however, their effect on airway electrogenic Na^+-K^+ activity is unknown. Previous findings from this and other laboratories have demonstrated that hyperpolarization elicited with pump stimulation is associated with tracheal smooth muscle relaxation (Gunst & Stropp, 1988; Schramm & Grunstein, 1989; 1995; Tamaoki *et al.*, 1994). In an extension of these observations, the present study was designed to determine: (1) whether corticosteroids modulate Na^+-K^+ pump activity in isolated airway segments. In the light of data suggesting an age-dependent reduction in the electrogenic contribution of the airway Na^+-K^+ pump (Souhrada *et al.*, 1988), the present study also investigated: (2) whether functional activity of the airway electrogenic Na^+-K^+ pump varies during post-natal development; and (3) whether corticosteroids modulate Na^+-K^+ pump activity in an age-dependent manner.

Methods

Tracheal and central bronchial segments of 5-10 mm length were isolated from maturing and adult New Zealand White rabbits killed by systemic air embolism following anaesthesia with xylazine (9 mg kg^{-1}) and ketamine hydrochloride (50 mg kg^{-1}). The airway segments were cleaned of loose connective tissue and suspended longitudinally between stainless steel triangular supports in siliconized Harvard 14- and 20-ml organ baths. The lower support was secured to the base of the water bath; the upper support was attached via gold chain to a Grass FT.03C force transducer from which isometric tension was continuously displayed on a multichannel recorder (SensorMedics). Care was taken not to injure the epithelium and to place the membranous portion of the trachea between the supports in order to maximize the recorded tension generated by the contracting trachealis muscle. The tissues were bathed in modified Krebs-Ringer solution of pH 7.3-7.4 and composition (in mM): NaCl 125, $NaHCO_3$ 14, KCl 4, $CaCl_2.2H_2O$ 2.25, $MgSO_4.7H_2O$ 1.46, $NaH_2PO_4.H_2O$ 1.2 and glucose 11. The baths were aerated with 5% CO_2 in O_2, at a constant temperature of 37°C.

The tracheal (TSM) and bronchial (BSM) segments were allowed to equilibrate in the organ baths for 1 h, during which time each was passively stretched on several occasions to a

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tension of 4 to 8 g, depending on the size of the tissue. The resting tensions of the immature and adult tissues were then set to optimize the resting length of each airway muscle segment. The latter was previously determined by assessing the tissues' maximal contractile responses to a standard electrical field stimulus (15 V; 2 ms pulse duration; 50 Hz stimulation frequency; 24.5 mA) delivered by a Grass S48 stimulator via 0.3 cm^2 platinum plate electrodes. The tension necessary to achieve the optimal resting length amounted to approximately 10–20 g g^{-1} tissue.

First, contractility to cholinoceptor stimulation was measured in all TSM and BSM segments by cumulative administration of methacholine in final bath concentrations ranging from 0.001 to 100 μM. Following thorough rinsing, activity of the airway Na^+-K^+ pump was assessed physiologically by the technique of Webb & Bohr (1978). The Na^+-K^+ pump was initially inactivated by incubating tissues for 1 h in K^+-free buffer, made isotonic with the regulatory buffer by substitution of NaCl for KCl. Exposure to K^+-free buffer elicited no significant contractions in the rabbit airway segments. Thereafter, each tissue was contracted half maximally with an individualized concentration of methacholine (i.e., EC_{50} concentration). During the sustained contraction to cholinoceptor stimulation, the Na^+-K^+ pump was progressively reactivated by cumulative re-addition of KCl (0.001 to 65 mM). Following rinsing with K^+-free buffer and re-establishment of baseline tension, half the airway segments were exposed to 500 μM methylprednisolone for 1 h. This concentration and incubation period were chosen from preliminary studies in newborn and adult TSM which demonstrated: (1) a modest potentiation of KCl-induced relaxation by 100 μM methylprednisolone (approximately one-third of 500 μM response); (2) no further potentiation by 1000 μM methylprednisolone; and (3) no difference in 500 μM response with 1 h or 2 h exposure. The tissues were again half-maximally contracted with methacholine, and the functional activity of the pump was then re-determined with the repeat administration of KCl in the absence (control) and presence of methylprednisolone. Separate studies were designed to demonstrate the dependence and specificity of the relaxant KCl response to activation of the Na^+-K^+ pump. In these paired studies, the effects of KCl administration were examined in the following conditions: (1) the absence and presence of the Na^+-K^+ ATPase pump inhibitor, ouabain (50 μM, 30- to 45-min pretreatment); (2) the absence and presence of the β-adrenoceptor antagonist, propranolol (10 μM, 30-min pretreatment), or the cyclo-oxygenase inhibitor, indomethacin (10 μM, 45-min pretreatment); and (3) the absence and presence of the respiratory epithelium (removed by abrasion with a cotton-tipped applicator). All relaxations to KCl were compared to the maximal relaxation of the cholinoceptor-induced contraction elicited by atropine (100 μM), and were expressed as a percentage of the atropine relaxation.

At the end of each experiment, the airway segments were blotted on a gauze pad and weighed. All tensions were normalized and expressed as grams of active tension per gram tissue weight. Relaxations were analysed as a percentage reversal of the methacholine contraction. In characterizing the dose-response curves, both the maximal response (i.e., R_{max}) and the negative logarithm of the concentration of agonist associated with 50% of R_{max} (i.e., $pC_2 = -\log EC_{50}$) were determined. Results are expressed as mean ± s.e. values of the number of observations (n). Statistical analyses were performed by Student's two-tailed paired or unpaired t tests or by analysis of variance (ANOVA), where appropriate. A P value of ≤ 0.05 was considered to be significant.

All drugs employed in this study (i.e., methacholine, methylprednisolone, atropine, propranolol, and indomethacin) were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Drug doses are expressed as final bath concentrations. The methylprednisolone was prepared as a 0.05 M stock solution in dimethyl sulphoxide (Sigma) to provide final bath concentrations of 500 μM. Previous control experiments demonstrated that the dose of dimethyl sulphoxide delivered with administration of the steroid solutions (i.e., 1% v v^{-1}) exerted no systematic effect on TSM tension (Schramm & Grunstein, 1989).

Results

Maturation of functional Na^+-K^+ pump activity in rabbit TSM and BSM

Following initial incubation in K^+-free buffer, the subsequent cumulative administration of KCl induced dose-dependent relaxation of TSM and BSM segments half-maximally contracted with methacholine, as demonstrated for a representative adult TSM ring in Figure 1a. This KCl-induced relaxation was due to activation of the airway Na^+-K^+ pump, as the response was completely ablated in the presence of the pump inhibitor, ouabain (50 μM) (Figure 1b). We have previously demonstrated that ouabain does not prevent TSM from relaxing in response to other agents, as the relaxant responsiveness to a cyclic AMP analogue was not affected by similar ouabain pretreatment (Schramm & Grunstein, 1995). In separate studies, pretreatment with propranolol (10 μM) or indomethacin (10 μM) had no effect on subsequent KCl-mediated TSM relaxation (Figure 2a; $P = 0.25$ by repeated measures ANOVA), indicating that the relaxant response was not associated with any KCl-induced release of noradrenaline or relaxant prostaglandins. Nor was the response to KCl affected by epithelial removal (Figure 2b; $P = 0.80$), demonstrating that KCl did not stimulate release of any epithelial-derived relaxing factor(s) that could have contributed to the relaxant response.

Initial studies demonstrated significant ontogenetic but no airway topographical differences in responsiveness to KCl. Neither the R_{max} response nor the sensitivity to KCl (represented by the pC_2 concentration) was significantly different in TSM and BSM isolated from 7 newborn (i.e., 1–2 week) and 6 adult rabbits. The mean ± s.e. R_{max} response amounted to 63.4 ± 7.6% and 61.2 ± 6.8% in newborn TSM ($n = 18$) and BSM ($n = 13$; $P = 0.84$), respectively, and 51.3 ± 6.8% and 41.0 ± 7.7% in adult TSM ($n = 24$) and BSM ($n = 12$; $P = 0.36$). KCl sensitivity averaged 2.07 ± 0.07 and 2.33 ± 0.26 −log M in newborn TSM and BSM, respectively, compared to 2.40 ± 0.13

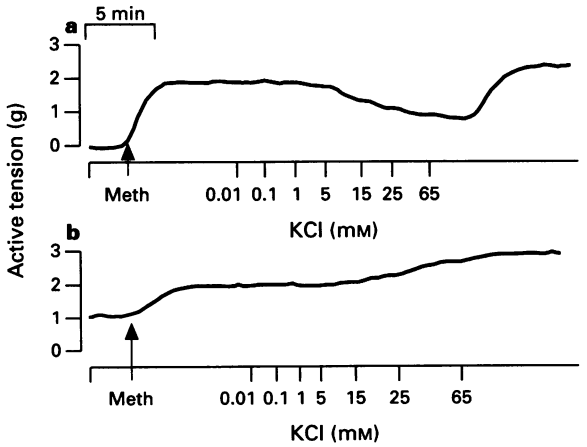

Figure 1 Representative tracing of KCl-induced relaxation of tracheal smooth muscle (TSM) isolated from an adult rabbit. (a) Re-addition of KCl to TSM half-maximally contracted with methacholine (meth) in K^+-free buffer elicits dose-dependent relaxation of approximately 50% of the cholinoceptor-induced contraction. (b) In the presence of ouabain (50 μM) the relaxation is completely abolished, and KCl re-addition elicits further contraction in the absence of airway Na^+-K^+ pump activity. The active tension present prior to methacholine adminstration is due to contraction elicited during pretreatment with ouabain.

and 2.08±0.09 −log M in adult TSM and BSM ($P=0.28$ and 0.10). Accordingly, data from TSM and BSM were pooled to determine age-dependent differences in functional Na^+-K^+ pump activity in airway smooth muscle (ASM; Figure 3). Activation of the Na^+-K^+ pump with KCl resulted in mean±s.e. maximal relaxation of 47.8±5.2% of the methacholine-induced contraction in the adult ASM. Relative to this adult response, pump activation in the newborn ASM elicited significantly greater relaxation, to 62.5±5.2% of the methacholine contraction ($P=0.040$ by 2-factor ANOVA). In contrast, no difference was observed in airway pump sensitivity to KCl, as reflected by the mean±s.e. pC_2 concentrations of 2.18±0.12 and 2.29±0.09 −log M in the newborn and adult ASM, respectively ($P=0.79$).

In parallel to their enhanced maximal relaxant response to KCl, newborn ASM segments were significantly more sensitive to methacholine than adult tissues, with mean±s.e. pC_2 values of 5.81±0.009 and 5.89±0.07 −log M in newborn TSM and BSM, vs. 5.38±0.07 and 5.02±0.15 in adult TSM and BSM, respectively ($P\leqslant 0.0001$, 2-factor ANOVA). No systematic topographical differences were observed in TSM vs. BSM cholinoceptor sensitivity ($P=0.13$, 2-factor ANOVA). In comparing the methacholine and KCl responses, the ratio of KCl-induced R_{max} to methacholine pC_2 concentration (in −log μM) was determined in ASM from newborn, 5-week-old, and adult rabbits. This R_{max}/pC_2 ratio averaged (mean±s.e) 11.1±0.9, 7.6±1.0, and 9.5±1.2% $(-\log M)^{-1}$ in the newborn, 5-week, and adult ASM, respectively ($P=0.18$ by ANOVA) and, thus, appeared to remain constant despite ontogenetic changes in both cholinoceptor sensitivity and pump responsiveness in the tissues. In addition, no correlation was found between the degree of cholinoceptor-induced precontraction and the subsequent R_{max} response to KCl for tissues of varying maturational age contracted to between 40 and 65% of maximal methacholine-induced tension (correlation coefficient $r=-0.211$; $P=0.17$).

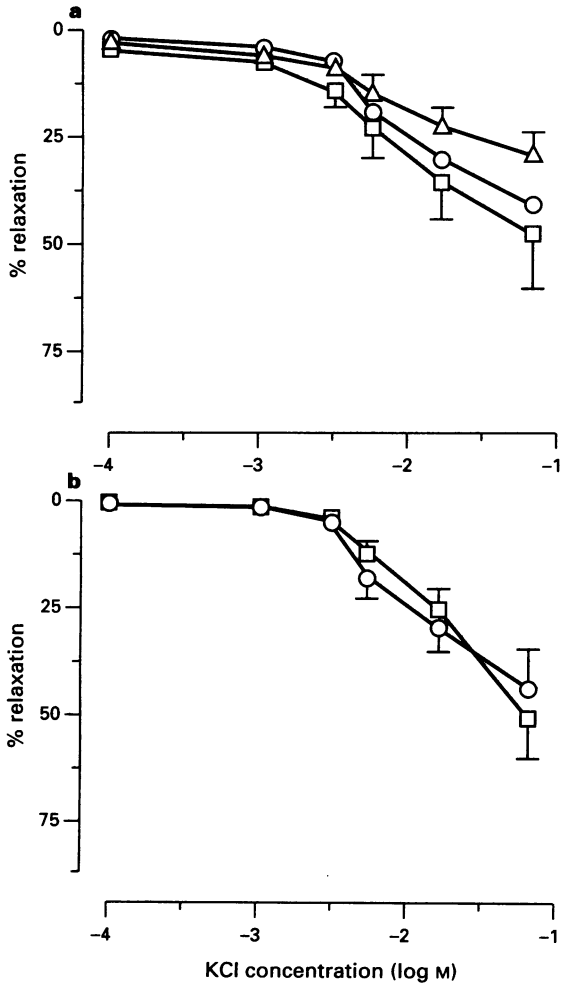

Figure 2 Comparison of relaxant dose-response relationships to KCl in control and treated adult TSM. (a) No significant differences are observed in the relaxant response to KCl in TSM pretreated with propranolol (△) or indomethacin (□), relative to paired control tissues (○; $n=10$ in each group). (b) No significant difference is observed in the relaxant response to KCl in epithelial-denuded TSM (□) compared to epithelial-intact control tissues (○; $n=10$). Data are expressed as percentage reversal of half-maximal cholinergic contraction, and are plotted as mean±s.e. values. Data are expressed as mean±s.e. values.

Ontogenetic effect of methylprednisolone on airway Na^+-K^+ pump activity

At each age, the initial (pre-steroid) relaxant responses to airway Na^+-K^+ pump activation with KCl were similar in the paired control tissues and the ASM segments subsequently treated with methylprednisolone. Moreover, no significant differences were found between the first and second control KCl relationships. In contrast, after 1 h incubation in K^+-free buffer containing 500 μM methylprednisolone, the relaxant response to KCl was significantly potentiated in ASM segments from both newborn and adult rabbits (Figure 4). In the newborn airways, both maximal relaxation and sensitivity to KCl were significantly increased following steroid treatment (R_{max}: 98.1±3.1% vs. 55.7±5.9%, $P\leqslant 0.0005$; pC_2: 2.99±0.30 vs. 2.23±0.15 −log M, $P=0.0043$). In adult ASM, however, only the maximal relaxant response was increased (R_{max}: 73.8±6.3% vs. 50.3±6.4%, $P\leqslant 0.0005$; pC_2: 2.29±0.10 vs. 2.29±0.10 −log M, $P=0.97$). In contrast to the effect on the airway relaxant response to KCl, corticosteroid treatment did not influence the ASM contractile responses to methacholine.

We further compared the ontogeny of the effect of methylprednisolone on Na^+-K^+ pump activity in ASM from newborn, 5-week-old, and adult rabbits. Figure 5a depicts the differences between the first and second R_{max} responses to KCl in steroid-treated and control tissues (i.e., $\Delta R_{max} = R_{max}(2) - R_{max}(1)$). For all 3 age groups, the ΔR_{max} values were significantly enhanced ($P\leqslant 0.0001$) following methylprednisolone, and the latter corticosteroid effect was relatively more pronounced in the immature vs. mature ASM, although the

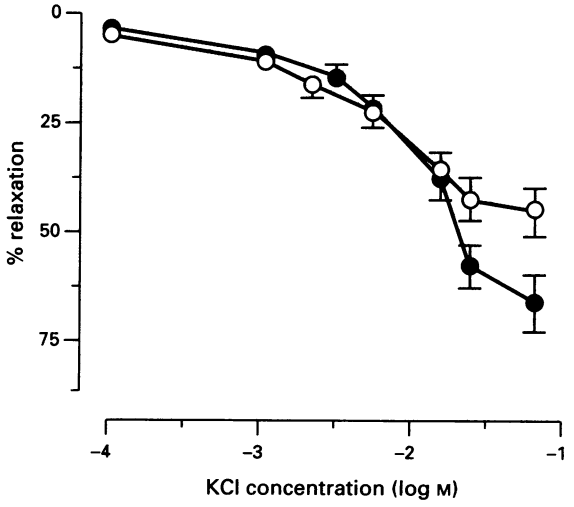

Figure 3 Comparison of airway smooth muscle (ASM) relaxant dose-response relationships to KCl in newborn and adult airway segments. Relative to responses in adult tissues (○, $n=36$), newborn airways (●, $n=31$) depicted significantly increased maximal relaxation but no change in sensitivity to KCl. Data represent mean±s.e. values.

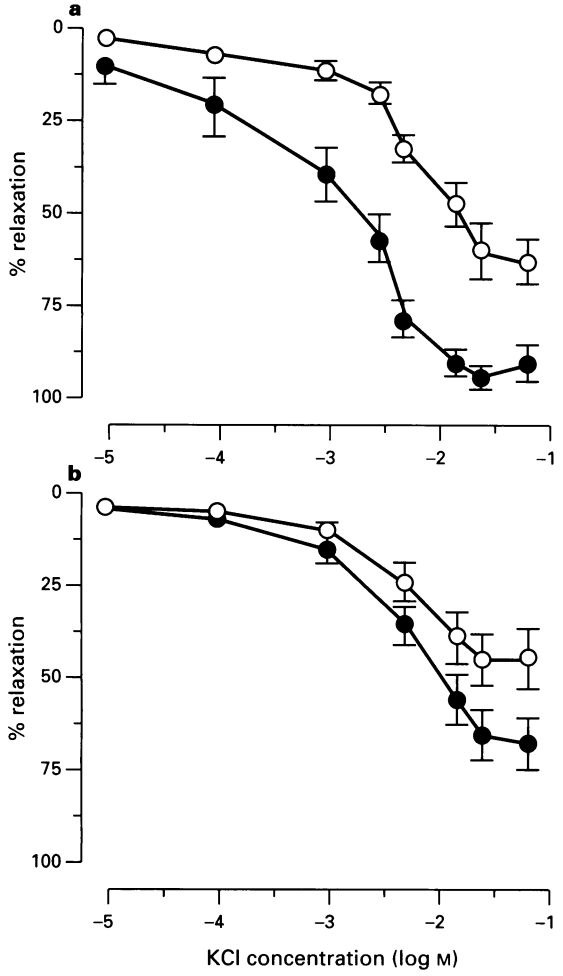

Figure 4 Comparison of relaxant dose-response relationships to KCl in newborn and adult ASM before and after methylprednisolone. (a) Relative to pre-steroid treatment (○), methylprednisolone (500 μM × 1 h) elicited increases in both maximal relaxation and sensitivity to KCl in newborn ASM segments (●; $n=22$). (b) Relative to pre-steroid treatment (○), methylprednisolone increased maximal relaxation to KCl only in adult ASM segments (●; $n=23$). Data represent mean ± s.e. values.

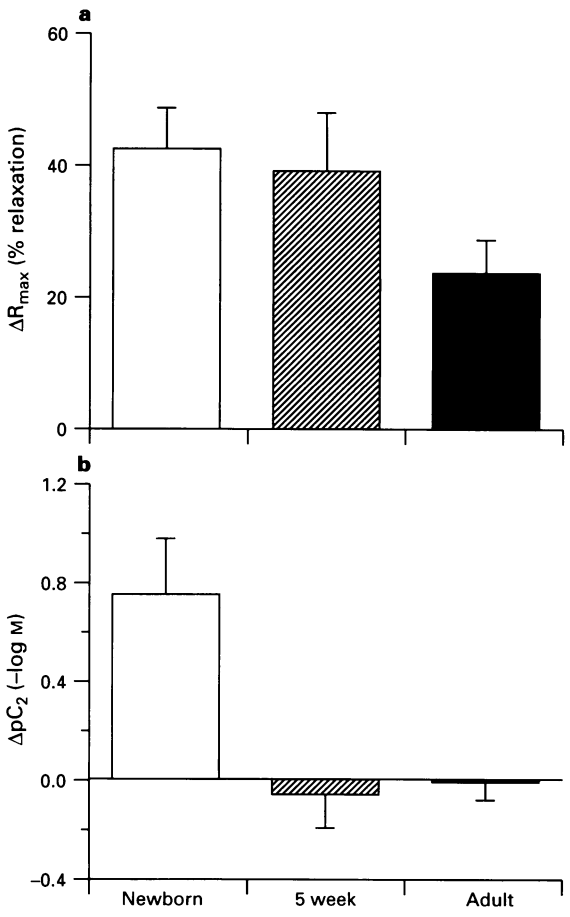

Figure 5 Comparison of methylprednisolone potentiation of Na^+-K^+ pump activity in airway segments from newborn ($n=22$, open columns), 5-week ($n=9$, hatched columns), and adult ($n=23$, solid columns), rabbits. (a) Change in maximal KCl relaxation (ΔR_{max}) elicited after methylprednisolone treatment, determined as $\Delta R_{max} = R_{max}(2) - R_{max}(1)$ and expressed as percentage reversal of the methacholine contraction. For all 3 age groups, methylprednisolone significantly enhanced ΔR_{max}, and the latter effect was relatively more pronounced in immature ASM. (b) Change in KCl sensitivity after methylprednisolone treatment, determined as $\Delta pC_2 = pC_2(2) - pC_2(1)$. Methylprednisolone significantly increased ASM sensitivity to KCl only in the newborn tissues. Data are expressed as mean ± s.e. values.

trend did not reach statistical significance for the 3 groups ($P=0.071$; 2-factor repeated measures ANOVA). Figure 5b illustrates the corresponding differences in the pC_2 values to KCl obtained in the same tissues, wherein $\Delta pC_2 = pC_2(2) - pC_2(1)$. Methylprednisolone treatment significantly increased ASM sensitivity to KCl only in the newborn tissues ($P=0.0026$; 2-factor repeated measures ANOVA), with a 5.7 fold reduction in the geometric mean EC_{50} value for KCl.

Discussion

To supplement basic electrophysiological measurements, a variety of methods have been developed for assessing Na^+-K^+ pump activity. One physiological approach, as described by Webb & Bohr (1978), quantitates pump activity in terms of ouabain-inhibitable, K^+-induced relaxation of smooth muscle contracted in K^+-free buffer. The latter relaxation is accompanied by membrane hyperpolarization and is attributed to reactivation of smooth muscle Na^+-K^+ pump previously inactivated in the K^+-free buffer. Intracellular Na^+ content increases during the period of pump inactivation, and this Na^+ 'loading' drives the reactivation of the Na^+-K^+ pump with external K^+ replenishment. This methodology provides an indirect assessment of Na^+-K^+ pump activity; however, studies have demonstrated good qualitative correlations between pump activity determined by the Webb & Bohr method and other techniques, including ^{86}Rb uptake (Marin *et al.*, 1988; Muller *et al.*, 1989; Shubat 1990), maximal [^{3}H]-ouabain binding (Marin *et al.*, 1988), and direct Na^+-K^+ ATPase activity (Muller *et al.*, 1989).

One advantage to the Webb & Bohr technique is that it provides a quantitative estimate of the functional relaxant influences of ASM Na^+-K^+ pump activity. The K^+-induced relaxation of ASM was specifically inhibited by ouabain (Figure 1; Schramm & Grunstein, 1989), and it did not include the release of bronchodilator catecholamines, prostaglandins, or epithelial-derived factors, since the relaxation was unaffected by propranolol, indomethacin or epithelial removal, respectively (Figure 2). We have also noted no difference in the KCl-induced relaxation in TSM pretreated with the calcium-channel blocker, nifedipine (Schramm & Grunstein 1989). Thus, while the potential contribution from other transmitters or ion transport mechanisms cannot be completely ruled out, our findings are comparable to those previously reported using the same method to assay maximal physiological Na^+-K^+ pump activity in vascular (Feletou & Vanhoutte, 1988; Marin

et al., 1988; Rinaldi & Bohr, 1989: Shubat *et al.*, 1990), gastrointestinal (Miasiro *et al.*, 1985; Muller *et al.*, 1989), and airway smooth muscle (Souhrada & Souhrada 1981; Gunst & Stropp, 1988; Schramm & Grunstein, 1989: Schramm & Grunstein, 1995).

The sensitivity of the airway electrogenic Na^+-K^+ pump to $[K^+]_e$ varied neither topographically, as noted above, nor ontogenetically. Half maximal ASM relaxations were elicited with geometric mean KCl concentrations of 6.2 and 5.1 mM in the newborn and adult tissues, respectively. The latter KCl EC_{50} values are similar to the geometric mean EC_{50} concentrations of 7.9 and 7.0 mM previously reported by us in adult rabbit TSM (Schramm & Grunstein, 1989; 1995). Moreover, these concentrations are in accord with the $[K]_e$ EC_{50} values for pump activation of 5 mM determined physiologically in vascular smooth muscle (Webb & Bohr, 1978), and 4.7 mM determined electrophysiologically in intestinal smooth muscle (Droogmans & Casteels, 1976).

In contrast to the above relative constancy in KCl sensitivity, the maximal relaxant response to Na^+-K^+ pump activation with KCl was significantly reduced with age (Figure 3), decreasing from 62.5% in the newborn tissues to 40.5% and 47.8% in the 5-week and adult ASM, respectively. This age-related attenuation in maximal responsiveness cannot be attributed to differences in level of cholinoceptor-induced contraction of the tissues, since the latter averaged 48.6%, 41.4%, and 47.6% of maximal cholinoceptor-induced contraction in the three age groups, respectively. If methacholine influenced the Na^+-K^+ pump activity directly, independent of its contractile effects on the ASM, the subsequent K^+-induced relaxation might not accurately reflect physiological pump activity (Droogmans & Casteels, 1976). The latter mechanism is unlikely, however, since cholinoceptor agonists have not been shown to modulate Na^+-K^+ ATPase activity (Gick *et al.*, 1988). Moreover, varying doses of methacholine associated with 40–65% of maximal cholinoceptor-induced contraction neither augmented nor inhibited K^+-mediated ASM relaxation in any age group.

The maturational decrease in KCl responsiveness may be due, at least in part, to an age-dependent reduction of Na^+-K^+ pump sites. The KCl R_{max} response was approximately 30% less in adult than newborn ASM. Similarly, the amount of ouabain-inhibitable E_m decreases ~45% with maturation in bovine trachealis muscle (Souhrada *et al.*, 1988). The difference in magnitude of these two ontogenetic trends may be partly related to inter-species variation but also to the tendency for measurements of ouabain-induced E_m changes to overestimate the electrogenic component of the Na^+-K^+ pump, due to secondary effects on other electrochemical ion gradients that contribute to the E_m. Such an ontogenetic decrease in airway Na^+-K^+ pump activity may represent a physiological adaptive response to maturational changes in airway contractility. In support of this concept, it has been reported that the ouabain-inhibitable component of E_m is enhanced in actively and passively sensitized guinea-pig TSM (Souhrada & Souhrada, 1984), and significant correlations have been observed between guinea-pig airway sensitivity to ouabain and histamine (Agrawal & Hyatt, 1986).

Thus, the Na^+-K^+ pump may exert a homeostatic mechanism in hyperreactive airways, with increased Na^+-K^+ ATPase activity serving to attenuate Na^+ and Ca^{2+} loading of the hyperreactive ASM cells (Agrawal & Hyatt, 1986). This speculation is supported by our finding that airway Na^+-K^+ pump activity is greater in newborn ASM, which also depicts an increased sensitivity to methacholine. In this connection, it is relevant to note that our observed maturational changes in maximal pump responsiveness were proportional to changes in airway cholinoceptor sensitivity, such that the R_{max}/pC_2 ratio remained constant during maturation. The latter finding provides compelling new evidence that airway Na^+-K^+ activity is coupled to the tissue's reactivity to bronchoconstrictor stimuli, a phenomenon probably reflecting the compensatory mechanism hypothesized above.

Apart from the relationship between Na^+-K^+ pump activity and cholinoceptor sensitivity, we found that resting airway Na^+-K^+ pump activity was potentiated by the acute administration of methylprednisoline, and that the latter effect varied in an age-dependent manner. In adult and 5-week ASM, maximal responsiveness was increased by 47% and 95%, respectively, after methylprednisolone administration, whereas pump sensitivity to KCl was unaltered (Figure 5). In contrast, steroid treatment of newborn ASM increased maximal responsiveness to KCl by 103% but also increased sensitivity to KCl by 5.7 fold. The mechanism(s) underlying the newborn tissues' greater response to corticosteroids remains to be identified. It is unlikely that these maturational differences are related to developmental regulation of isoform expression of the α catalytic subunit of Na^+-K^+ ATPase in ASM. In rat whole lung and epithelial preparations, expression of the α_1 isoform varies perinatally but predominates at all ages, and there is virtually no expression of the α_2 and α_3 isoforms (Orlowski & Lingrel, 1988; O'Brodovic *et al.*, 1993). Specific studies of α isoform expression have not been performed in ASM; however, the *in situ* localization of Na^+-K^+ ATPase mRNA in developing and maturing mouse lung revealed very weak hybridization signals with the α_2 and α_3 isoforms throughout the lung - including the airway smooth muscle (Crump *et al.*, 1995).

The acute response to methylprednisolone observed in rabbit ASM was too rapid to be due to glucocorticoid-induced transcription of pump enzymes. More likely, since KCl is a cofactor for Na^+-K^+ pump activation, the change in sensitivity to KCl administration represents a methylprednisolone-induced alteration in the kinetic characteristics of the Na^+-K^+ ATPase in newborn ASM. We have observed that β-adrenoceptor agonists also increase TSM relaxant sensitivity to KCl, by a factor of 3.5 (Schramm & Grunstein, 1995). It is likely that these hormones act on other sites or cofactors that are independently stimulatory to Na^+-K^+ ATPase, as suggested in other tissues, and that these other factors vary maturationally. The direct and/or indirect stimulation of Na^+-K^+ ATPase activity could account for the enhanced maximal relaxant response to KCl. Moreover, higher levels of potentiation may demonstrate a synergistic effect with extracellular $[K^+]_e$, such that Na^+-K^+ ATPase activity is enhanced at all levels of $[K^+]_e$ and the apparent affinity for KCl-induced ASM relaxations is increased.

To date, corticosteroid modulation of Na^+-K^+ pump activity has been most extensively studied in renal epithelium. In this tissue, dexamethasone elicits a glucocorticoid-specific increase in Na^+,K^+-ATPase activity by 40–60% within 2 h of administration (Rodriguez *et al.*, 1981; Rayson & Gupta, 1985). This degree of enhancement is similar to the potentiation of pump R_{max} activity observed in the present study in adult rabbit ASM. Additional observations on isolated renal tubules, as well as proximal colonic epithelium, suggest that glucocorticoids modulate the Na^+-K^+ pump without affecting $[Na^+]_i$ (Garg et al., 1985; Sellin & DeSoignie, 1985), although concomitant steroid-induced increase in $[Na^+]_i$ may exert a permissive effect on Na^+-K^+ pump activity (Rayson & Gupta, 1985). This potentiating effect of corticosteroids on the renal pump was ablated when dexamethasone was added to isolated, broken cell membrane preparations (Sinha *et al.*, 1981), indicating that corticosteroids augment Na^+,K^+-ATPase activity via an indirect mechanism. Such indirect mechanisms could include influencing intracellular concentrations of ions other than Na^+ or altering the plasma membrane composition of lipids stimulatory and/or inhibitory to the Na^+-K^+ pump (Melby *et al.*, 1981).

In conclusion, the present study examined the effect of corticosteroid treatment on the ontogeny of Na^+-K^+ pump activity in rabbit isolated airway smooth muscle. Our findings demonstrate that: (1) activation of the Na^+-K^+ pump elicits relaxation of cholinoceptor-induced airway segments; (2) the functional activity of the airway electrogenic Na^+-K^+ pump decreases with postnatal maturation in the rabbit; (3) methylprednisolone treatment potentiates the Na^+-K^+ pump

activity in rabbit ASM; and (4) the latter effect of the corticosteroid is relatively enhanced in immature airways. To the extent that the magnitude of membrane polarization may regulate the degree of ASM contractility, the present findings on the effects of methylprednisolone on the airway electrogenic Na^+-K^+ pump may, at least in part, account for the known efficacy of corticosteroid therapy in asthma.

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References

AGRAWAL, K.P. & HYATT, R.E. (1986). Airway responses to inhaled ouabain and histamine in conscious guinea pigs. *J. Appl. Physiol.*, **60,** 2089–2093.

CRUMP, R.G., ASKEW, G.R., WERT, S.E., LINGREL, J.B. & JOINER, C.H. (1995). In situ localization of sodium-potassium ATPase mRNA in developing mouse lung epithelium. *Am. J. Physiol.*, **269,** L299–L308.

DROOGMANS, G. & CASTEELS, R. (1976). Membrane potential and ion transport in smooth muscle cells. In *Physiology of Smooth Muscle.* ed. Bülbring, E. & Shuba, M.F. p. 438. New York: Raven.

FELETOU, M. & VANHOUTTE, P.M. (1988). Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br. J. Pharmacol.*, **93,** 515–524.

GARG, L.C., NARANG, N. & WINGO, C.S. (1985). Glucocorticoid effects on Na-K-ATPase in rabbit nephron segments. *Am. J. Physiol.*, **248,** F487–F491.

GICK, G.G., ISMAIL-BEIGI, F. & EDELMAN, I.S. (1988). Overview: hormonal regulation of Na,K-ATPase. In *The Na^+,K^+ Pump*, Part B: *Cellular Aspects*, ed. Skou, J.C., Norby, J.G., Maunsbach, A.B. & Esmann, M. pp. 277–295. New York: Alan R. Liss.

GUNST, S.J. & STROPP, J.Q. (1988). Effect of Na-K adenosinetrophosphatase activity on relaxation of canine tracheal smooth muscle. *J. Appl. Physiol.*, **64,** 635–641.

KAJI, D.M., THAKKAR, U. & KAHN, T. (1981). Glucocorticoid-induced alterations in the sodium pump of the human erythrocyte. *J. Clin. Invest.*, **68,** 422–430.

MARIN, J., SANCHEZ-FERRER, C.F. & SALAICES, M. (1988). Effects of ouabain on isolated cerebral and femoral arteries of the cat: a functional and biochemical study. *Br. J. Pharmacol.*, **93,** 43–52.

MATTHEWS, E.K., DROOGMANS, G. & HENDRICKS, H. (1973). Active ion transport and resting potential in smooth muscle cells. *Phil. Trans. R. Soc.* (Series B), **265,** 47–56.

MATTHEWS, E.K. & SUTTER, M.C. (1967). Ouabain-induced changes in the contractile and electrical activity, potassium content and response to drugs, of smooth muscle cells. *Can. J. Physiol. Pharmacol.*, **45,** 509–520.

MELBY, J.M., WENHOLD, A.R. & NELSON, D.H. (1981). Corticosteroid-induced lipid changes in rat liver microsomes. *Endocrinology*, **109,** 920–923.

MIASIRO, N., PAIVA, T.B., PEREIRA, C.C. & SHIMUTA, S.I. (1985). Reactivity to bradykinin and potassium of the isolated duodenum from rats with genetic and renal hypertension. *Br. J. Pharmacol.*, **85,** 639–646.

MULLER, M.J., HUIZINGA, J.D. & COLLINS, S.M. (1989). Altered smooth muscle contraction and sodium pump activity in the inflamed rat intestine. *Am. J. Physiol.*, **257,** G570–G577.

O'BRODOVICH, H., STAUB, O., ROSSIER, B.C., GEERING, K. & KRAEHENBUHL, J.P. (1993). Ontogeny of α_1 and β_1 isoforms of Na^+,K^+-ATPase in fetal distal rat lung epithelium. *Am. J. Physiol.*, **264,** C1137–C1143.

ORLOWSKI, J. & LINGREL, J.B. (1988). Tissue-specific and development regulation of rat Na,K-ATPase catalytic α isoform and β subunit mRNAs. *J. Biol. Chem.*, **263,** 10436–10442.

RAYSON, B.M. & GUPTA, R.K. (1985). Steroids, intracellular sodium levels, and Na^+/K^+-ATPase regulation. *J. Biol. Chem.*, **260,** 12740–12743.

RINALDI, G. & BOHR, D.F. (1989). Potassium-induced relaxation of arteries in hypertension: modulation by extracellular calcium. *Am. J. Physiol.*, **256,** H507–H512.

RODRIGUEZ, H.J., SINHA, S.K., STARLING, J. & KLAHR, S. (1981). Regulation of renal Na^+-K^+-ATPase in rat by adrenal steroids. *Am. J. Physiol.*, **241,** F186–F195.

SCHRAMM, C.M. & GRUNSTEIN, M.M. (1989). Mechanisms of protein kinase C regulation of airway contractility. *J. Appl. Physiol.*, 66, 1935–1941.

SCHRAMM, C.M. & GRUNSTEIN, M.M. (1995). Mechanism of protein kinase C potentiation of airway β-adrenergic relaxation. *Life Sci.*, **57,** 1163–1173.

SELLIN, J.H. & DESOIGNIE, R.C. (1985). Steroids alter ion transport and absorptive capacity in proximal and distal colon. *Am. J. Physiol.*, **249,** G113–G119.

SHUBAT, P.J., BOWERS, R.J. & HUXTABLE, R.J. (1990). Na^+/K^+-adenosine triphosphatase activity of pulmonary arteries after intoxication with the pyrrolizidine alkaloid, monocrotaline. *J. Pharmacol. Exp. Ther.*, **252,** 70–76.

SINHA, S.K., RODRIGUEZ, H.J., HOGAN, W.C. & KLAHR, S.C. (1981). Mechanisms of activation of renal (Na^+-K^+)-ATPase in the rat. Effects of acute and chronic administration of dexamethasone. *Biochem. Biophys. Acta*, **641,** 20–35.

SOUHRADA, M., ROTHBERG, K.G. & DOUGLAS, J.S. (1988). Membrane properties of bovine smooth muscle cells: effects of maturation. *Pulm. Pharmacol.*, **1,** 47–52.

SOUHRADA, M. & SOUHRADA, J.F. (1981). The direct effect of temperature on airway smooth muscle. *Respir. Physiol.*, **44,** 311–323.

SOUHRADA, M. & SOUHRADA, J.F. (1984). Immunologically induced alterations of airway smooth muscle cell membrane. *Science*, **225,** 723–725.

SOUHRADA, M., SOUHRADA, J.F. & CHERNIACK, R.M. (1981). Evidence for a sodium electrogenic pump in airway smooth muscle. *J. Appl. Physiol.*, **51,** 346–352.

TAMAOKI, J., TAGAYA, E., CHIYOTANI, A., YAMAWAKI, I. & KONNO, K. (1994). Role of K^+ channel opening and Na^+-K^+ ATPase activity in airway relaxation induced by salbutamol. *Life Sci.*, **55,** 217–223.

WEBB, R.C. & BOHR, D.F. (1978). Potassium-induced relaxation as an indicator of Na^+-K^+ ATPase activity in vascular smooth muscle. *Blood Vessels*, **15,** 198–207.

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