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Mechanisms of intrinsic tone in bullfrog lung: relaxant effects of indomethacin, ouabain and potassium

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1 Bullfrog hemilungs showed minimal relaxation ($9 \pm 2\%$ of the maximal relaxant effect of theophylline, I_{max}) after a 16 h incubation in 10^{-5} M indomethacin, indicating that prostaglandin synthesis plays little or no role in the high intrinsic tone characteristic of this preparation.

2 A higher concentration of indomethacin (10^{-4} M) produced greater relaxation ($23 \pm 3\%$ of I_{max}), but also markedly potentiated isoprenaline-induced relaxation. The interaction with isoprenaline was similar to that previously found for papaverine, a phosphodiesterase inhibitor.

3 Ouabain (10^{-5} and 10^{-4} M) produced an initial contraction followed by marked relaxation (50% of I_{max}), indicating that a ouabain-sensitive mechanism is of major importance in the maintenance of intrinsic tone.

4 Ouabain-treated hemilungs showed (a) reversal (relaxation) of the normal contractile response to 26 mM potassium and (b) marked impairment of the contractile response to calcium in calcium-depleted preparations. These effects suggest that ouabain-induced relaxation reflects a drug action on calcium movements.

5 The marked relaxation (30 to 40% of I_{max}) produced by 26 mM potassium in ouabain-treated hemilungs is of particular interest in that it indicates a mechanism of potassium-induced relaxation distinct from stimulation of sodium-potassium ATPase.

Introduction

Isolated preparations of airway smooth muscle vary greatly with respect to intrinsic tone (Main, 1964), i.e. the extent of contraction in the absence of exogenous chemical or physical stimuli. Tracheal and bronchial smooth muscle preparations obtained from rats have no measurable intrinsic tone (Jamieson, 1962; Burns & Doe, 1978), and most canine preparations show relatively little intrinsic tone (Yamaguchi *et al.*, 1976; Krell, 1978). In contrast, guinea-pig isolated tracheal muscle (Castillo & deBeer, 1947; Foster, 1960) and human bronchial muscle (Rosa & McDowall, 1951; Hawkins & Schild, 1951; Mathé *et al.*, 1971; Taylor *et al.*, 1984) are in the mid-range between maximal relaxation and maximal contraction, and the intrinsic tone is sufficient for dose-response relationships for bronchodilator drugs to be determined without addition of exogenous spasmogens.

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Except for studies relating intrinsic tone to prostaglandin synthesis, the mechanisms underlying intrinsic tone are obscure. In the guinea-pig tracheal muscle, synthesis of stimulant prostaglandins appears to be responsible for a large fraction of the high intrinsic tone, and indomethacin causes profound relaxation (Farmer *et al.*, 1972; 1974; Orehek *et al.*, 1973; 1975; Ono *et al.*, 1977), presumably by inhibition of the cyclo-oxygenase pathway. In contrast, indomethacin does not relax human bronchial preparations (Brink *et al.*, 1980; Hutás *et al.*, 1981), suggesting 'that the regulatory role of prostanoids in human airway muscle may be less than in other species' (Brink *et al.*, 1980).

Bullfrog lung is of particular interest with respect to intrinsic tone, in that the resting length of the excised lung is near maximal contraction (Downes & Taylor, 1983). This high level of intrinsic tone does not reflect the activity of intrapulmonary nerves since it is unaffected by atropine, ganglionic blocking agents, or tetrodotoxin (Downes & Taylor, 1983), and is enhanced rather than inhibited by local anaesthetics (Dow-

nes & Taylor, 1982). The present study tests the effects of indomethacin and ouabain, and shows that a ouabain-sensitive mechanism is far more important than prostaglandin synthesis to the maintenance of intrinsic tone in this preparation.

Methods

The lungs of pithed *Rana catesbeiana* (either sex, 100–500 g) were removed and divided longitudinally to yield four hemilungs. Each hemilung was mounted vertically under 2 g tension in a jacketed 50 ml bath containing a frog physiological solution, composition (mM): NaCl 89, $NaHCO_3$ 20, KH_2PO_4 2.5, $CaCl_2$ 2.0, $MgSO_4$ 1.5, glucose, 10. The ionic composition of this solution closely resembles that reported for frog plasma (Fenn, 1936; Conway, 1957). The bath solution was maintained at 15°C using a Lauda K-2/R circulator and thermoregulator. A mixture of CO_2 (1.3%) and O_2 (98.7%) was used to aerate the solution (Downes *et al.*, 1975), approximating the physiological values of CO_2 tension and blood pH (7.8) for intact, unanaesthetized bullfrogs at 15°C (Howell *et al.*, 1970).

In experiments requiring increased potassium concentrations, potassium was substituted for sodium to get a high (117.5 mM) potassium solution, composition (mM): KCl 95, $KHCO_3$ 20, KH_2PO_4 2.5, $CaCl_2$ 2.0, $MgSO_4$ 1.5, glucose 4. Aliquots of this high potassium solution, pre-cooled to 15°C, were added to the frog physiological solution to produce successively increasing potassium concentrations of 26, 44 and 81 mM without a change in the calcium or bicarbonate concentrations.

In experiments requiring incubation in calcium-free solutions, $CaCl_2$ was omitted from the frog physiological solution. Hemilungs were washed four times in the calcium-free solution at the start of the incubation period.

Changes in muscle length were recorded with isotonic transducers (Harvard 356) under a tension of 2 g. The lungs were allowed to equilibrate for 3 h before testing the drug effects. During this time, the lungs contracted until a stable resting length had been achieved. Previous studies (Taylor & Downes, 1982), have shown that this resting length is well maintained for several days, in the absence of drug treatment. In the present study, most experiments employed a 16 h (960 min) incubation period to assess drug effects, and matched hemilungs obtained from the same frogs were used as controls. At the end of the experiment, the hemilungs were washed and then maximally relaxed by incubation overnight with 10^{-2} M theophylline (Taylor & Downes, 1982). Preceding drug effects were expressed as a percentage of this maximal relaxant effect (I_{max}), i.e. '% maximal relaxation' (Figure 1). Negative values for % of I_{max} indicate that the drug produced a contraction rather than relaxation. A few experiments employed a two step treatment sequence in which the first treatment produced relaxation and the second treatment produced some degree of re-attainment of the initial resting length; in these experiments, the contractile effect of the second treatment was expressed as % re-attainment of resting length (Figure 6).

Data are presented as means ± s.e. of n determinations from different bullfrogs. Statistical evaluation was by either analysis of variance (more than 2 comparisons) or Student's t test. Data from 2 sets of hemilungs obtained from the same animals (matched hemilungs) were compared by the paired t test. Statistical significance was established at the $P < 0.05$ level.

Ouabain, indomethacin, isoprenaline, ascorbic acid and theophylline were purchased from Sigma Chemical Company (Milwaukie, WI). All drugs except indomethacin were dissolved in distilled water or frog physiological solution. Isoprenaline was prepared as a 10^{-2} M solution in water with 10^{-2} M ascorbic acid and 0.5 ml was added to a 50 ml bath. In experiments with isoprenaline, ascorbic acid was also added to the matched hemilungs so that all organ baths contained 10^{-4} M ascorbic acid. Indomethacin was dissolved in absolute ethanol and 125 µl of this ethanol solution was added to a 50 ml bath, which produced a final ethanol concentration of 4.3×10^{-2} M. Indomethacin effects were compared with those of the vehicle control (4.3×10^{-2} M ethanol) in matched hemilungs.

Results

Indomethacin

Incubation for 16 h with the vehicle control (4.3×10^{-2} M ethanol) produced no significant change in mean resting length, although many preparations underwent a slight contraction during the first hour. Matched hemilungs incubated with 10^{-5} M indomethacin were slightly, but significantly relaxed, when compared to the vehicle controls at 120, 240, 480 and 960 min (Table 1). Increasing the concentration of indomethacin to 10^{-4} M produced a slightly greater relaxation (Table 1), which after 16 h of incubation ($n = 10$) was equivalent to 23 ± 3 (± s.e.) % of I_{max}. The animals used in these studies varied in weight from 150 to 500 g, and there was no relationship between the effect of indomethacin (or lack of effect) and body weight.

Since the limited effect of indomethacin might reflect a gradual loss of indomethacin from the solution, 4 hemilungs from each of the above series

Table 1 % of maximal relaxant effect of theophylline 10^{-2} M (I_{max}) elicited by indomethacin 10^{-5} and 10^{-4} M and vehicle controls in matched hemilungs

	Time after drug addition (min)				
	60	*120*	*240*	*480*	*960*
Indomethacin 10^{-5} M ($n = 11$)	−2±1	0±1	3±1	7±1	9±2
Matched Controls ($n = 11$)	−2±1	−3±1	−2±1	−1±1	−4±2
P*	>0.05	<0.01	<0.01	<0.01	<0.01
Indomethacin 10^{-4} M ($n = 10$)	0±2	6±2	13±3	20±3	23±3
Matched Controls ($n = 10$)	−2±1	−1±1	0±1	−1±1	−4±2
P*	>0.05	<0.01	<0.01	<0.01	<0.01

Data shown are means ± s.e. Negative numbers indicate contraction rather than relaxation.
*Paired t test, indomethacin vs matched controls.

were incubated for an additional 6 h during which freshly prepared indomethacin solutions were added at hourly intervals. At the end of the initial 16 h incubation, relaxation was equivalent to 15 ± 4 ($n = 4$) and 22 ± 6% ($n = 4$) of I_{max}, in preparations exposed to 10^{-5} and 10^{-4} M indomethacin, respectively; after an additional 6 h and 6 changes of solution, relaxation in these preparations was equivalent to 17 ± 5 and 23 ± 6% of I_{max}, respectively.

In additional studies, the possible interactions of indomethacin with β-adrenoceptor-mediated pathways were assessed by incubating matched hemilungs with indomethacin (10^{-5} or 10^{-4} M), isoprenaline (10^{-4} M), or a combination of indomethacin and isoprenaline. Indomethacin 10^{-5} M did not alter the relaxant response to isoprenaline (Figure 1), whereas the higher concentration of indomethacin (10^{-4} M) markedly potentiated the isoprenaline-induced relaxation (Figure 2).

Ouabain

Ouabain (10^{-4} and 10^{-5} M) caused a transient contraction followed by a marked, progressive relaxation ($P < 0.01$) which peaked in 4 to 16 h. Since the resting length of untreated hemilungs is already near maximal contraction (Downes & Taylor, 1983), the ouabain-induced contraction was of much less magnitude than the eventual relaxation. Ouabain produced qualitatively similar effects in hemilungs obtained from both large (250–500 g), and small (100–150 g) bullfrogs, but the relaxant effect was significantly greater in the smaller frogs. In these ($n = 5$), 10^{-4} and 10^{-5} M ouabain produced 50 ± 6 and 50 ± 3% of I_{max}, respectively (Figure 3). Although the peak relaxant effects were identical at both concentrations, they occurred more rapidly (240 to 270 min) at the higher concentration than at the lower (630 to 750 min). Peak relaxant effects were usually followed by a partial

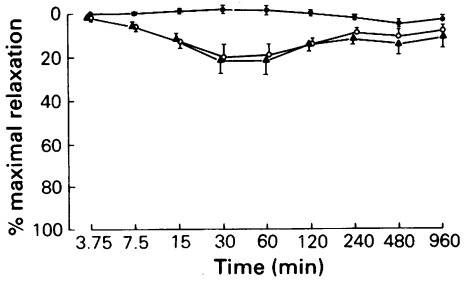

Figure 1 Lack of effect of 10^{-5} M indomethacin on the response to isoprenaline. Matched hemilungs from 5 frogs. Time (log scale) is from the addition of isoprenaline. Indomethacin has been added to the matched hemilungs 15 min previously. Mean responses in the presence of indomethacin 10^{-5} M (●), isoprenaline 10^{-4} M (○) and indomethacin 10^{-5} M plus isoprenaline 10^{-4} M (▲) are shown; vertical lines indicate s.e.

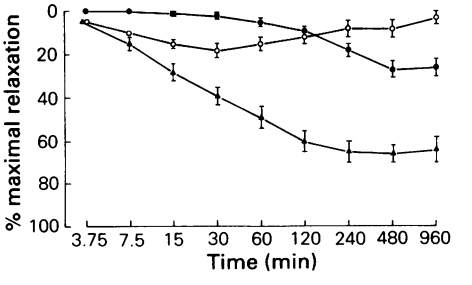

Figure 2 Potentiation of isoprenaline-induced relaxation by 10^{-4} M indomethacin. Matched hemilungs from 5 frogs. Time (log scale) is from the addition of isoprenaline. Indomethacin has been added to the matched hemilungs 15 min previously. Mean responses in the presence of indomethacin 10^{-4} M (●), isoprenaline 10^{-4} M (○) and indomethacin 10^{-4} M plus isoprenaline 10^{-4} M (▲) are shown; vertical lines indicate s.e.

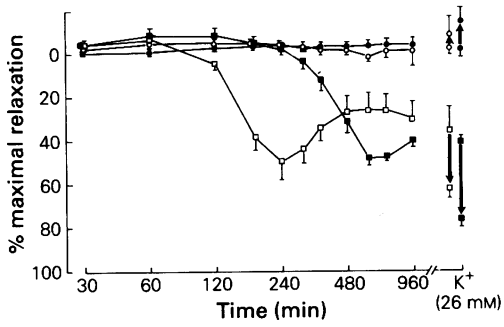

Figure 3 Time course (log scale) for ouabain-induced effects in bullfrog hemilungs. Matched hemilungs from 5 frogs, weighing 100 to 150 g. Potassium effects (shown on the right) were tested after completion of the 16 h incubation period. Length change produced by addition of 26 mM potassium is indicated by heavy arrows. Mean responses in the absence (control, ●) and presence of ouabain 10^{-6} M (○), 10^{-5} M (■), 10^{-4} M (□) are shown; vertical lines indicate s.e.

recovery of resting length (Figure 3). At 10^{-6} M ouabain failed to elicit significant relaxation even after 16 h of incubation.

In hemilungs obtained from larger frogs, ouabain 10^{-4} M ($n = 6$) and 10^{-5} M ($n = 4$) produced less ($P < 0.05$) intense relaxation (31 ± 4 and 29 ± 4% of I_{max}, respectively) and required a longer ($P < 0.05$) time for peak effects (270 to 570 min at 10^{-4} M and 960 min or more for 10^{-5} M).

Effect of potassium following ouabain-pretreatment

Hemilungs from the small frogs were subsequently employed to study the effect of changes in extracellular potassium concentration. Following completion of the 16 h incubation, the control and ouabain-treated hemilungs were exposed to increasing concentrations of potassium. Control hemilungs were contracted by 26 mM potassium ($P < 0.05$), further contracted and/or slightly relaxed by 44 mM potassium, and slightly relaxed ($P < 0.05$) by 81 and 117.5 mM potassium (Figures 4 and 5). Peak effects were obtained within 1 h of the change in potassium concentration. Potassium-induced responses were similar in hemilungs pretreated with 10^{-6} M ouabain, which like the control preparations had not undergone any significant relaxation during the preceding 16 h. In contrast, at the two higher concentrations of ouabain, which had relaxed the hemilungs, the normal contractile response to 26 mM potassium was reversed so that potassium elicited a marked relaxation ($P < 0.01$) (Figures 3–5). Relaxation was followed by partial recovery; although further increases in the potassium concentration (Figure 4) again induced relaxation, its peak effect did not exceed that produced by the initial exposure to 26 mM potassium. The extent of further relaxation produced by 26 mM potassium in the ouabain-relaxed hemilung was comparable to 30 to 40% of I_{max} (Figures 3 and 5). Repeated washing of the preparations in drug-free frog physiological solution rapidly reversed potassium-induced relaxation, but did not restore the pre-ouabain resting length.

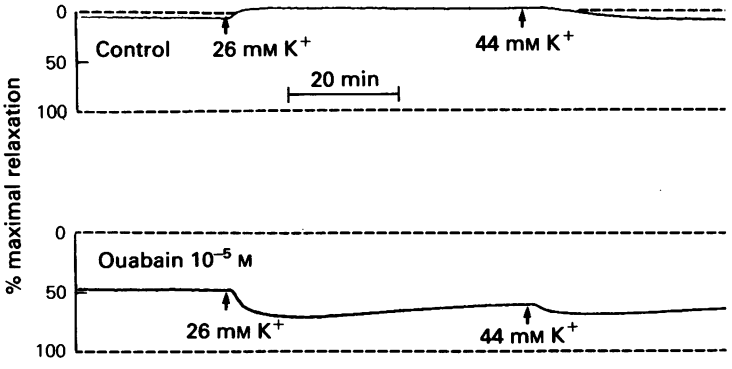

Figure 4 Potassium reversal in ouabain-treated preparations. Sample traces from data shown in Figures 3 and 5. In the control hemilung (top), after completion of the 16 h incubation, 26 mM potassium elicited only a contraction, whereas 44 mM potassium produced some degree of relaxation. In contrast, in the ouabain-treated hemilung (bottom), both concentrations produced only a relaxation of approximately equal magnitude. The potassium-induced relaxation was not well sustained, but could be restored by increasing the postassium concentration. Dotted lines at top and bottom show resting length at the start of the 16 h incubation and I_{max} at the completion of the experiment, respectively.

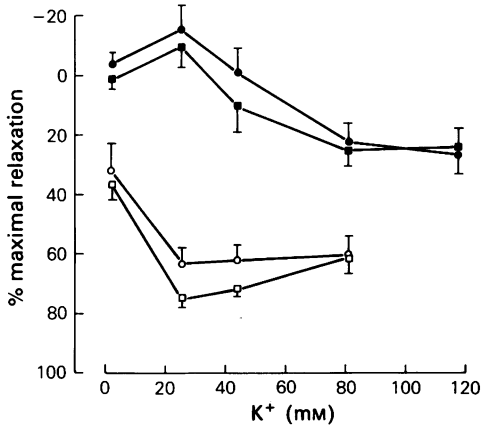

Figure 5 Dose-response curves for potassium-induced effects in control and ouabain-treated hemilungs. Data from the same experiments shown in Figures 3 and 4. Negative numbers indicate contraction rather than relaxation. Mean responses ($n = 5$) in the absence (control, ●) and presence of ouabain 10^{-6} M (■), 10^{-5} M (□), 10^{-4} M (○) are shown; vertical lines indicate s.e.

Effect of calcium following ouabain-pretreatment

In a further set of experiments, hemilungs ($n = 5$) were incubated for 16 h in a calcium-free solution, following which 2 mM $CaCl_2$ was added to the solution to restore a normal extracellular calcium concentration. The hemilungs slowly relaxed during incubation in the calcium-free solution, and contracted in response to addition of calcium. The extent of relaxation in calcium-free solution was significantly greater in the presence of ouabain (10^{-4} and 10^{-5} M) than in control hemilungs (Table 2). In control hemilungs, re-addition of calcium produced contraction over and above restoration of initial resting length. Ouabain induced a concentration-related depression of the contractile response to re-addition of calcium (Figure 6). In addition, the two higher ouabain concentrations induced a marked distortion of the time course for calcium-induced contraction which was greatly retarded and biphasic (Figure 6).

Discussion

Indomethacin

The concentrations of indomethacin that were employed in this study are comparable to or higher than those used in similar experiments with mammalian tissues (Vane, 1971; Orehek *et al.*, 1973; Farmer *et al.*, 1974) and are in the range shown to cause marked (>85%) inhibition of prostaglandin synthesis in amphibian tissues (6×10^{-6} M, Wong *et al.*, 1972; 5×10^{-5} M, Halushka *et al.*, 1980; 10^{-5} M, Ghiara *et al.*, 1984). In guinea-pig tracheal chains, the ED_{50} for inhibition of intrinsic tone by indomethacin is about 10^{-6} M (Farmer *et al.*, 1972) or slightly less (Ono *et al.*, 1977). In contrast, in bullfrog hemilungs 10^{-5} M indomethacin produced only a small effect (9% of I_{max}). Raising the concentration to 10^{-4} M produced greater relaxation (23% of I_{max}), but also markedly potentiated the effect of isoprenaline.

Normally, the maximal effect of isoprenaline and other β-adrenoceptor agonists in bullfrog hemilungs is relatively slight (20% of I_{max}) and occurs at, or below, 10^{-4} M concentration (Taylor & Downes, 1982; Dow-

Table 2 % of I_{max}* elicited by 16 h incubation of matched hemilungs in calcium-free solutions

	*Conc. ouabain present***		
Control	10^{-6} M	10^{-5} M	10^{-4} M
21±3	31±5	51±9	44±4
*P***	>0.05	<0.05	<0.05

Data shown are means ± s.e., $n = 5$.
*Maximal effect of theophylline 10^{-2} M
**Ouabain added at start of incubation.
***Analysis of variance with studentized range test.

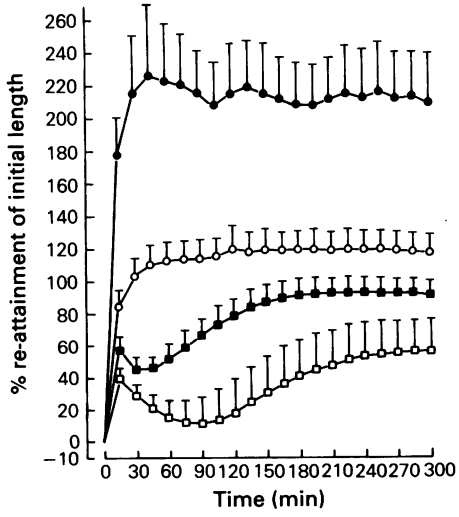

Figure 6 Ouabain-induced distortion of the contractile response to calcium (2 mM) in calcium-depleted preparations (16 h incubation in calcium-free solution). Mean responses ($n = 5$) in the absence (control, ●) and presence of ouabain 10^{-6} M (○), 10^{-5} M (■), 10^{-4} M (□) are shown; vertical lines indicate s.e.

nes & Taylor, 1983). However, the relaxant effect, as well as the increase in tissue cyclic AMP content, can be markedly potentiated by addition of papaverine (Taylor & Downes, 1982), an inhibitor of cyclic nucleotide phosphodiesterase. In the present study, indomethacin-induced potentiation of the effect of isoprenaline appears to be similar to that previously observed with papaverine, so that part of the relaxant effect of 10^{-4} M indomethacin may have been due to inhibition of phosphodiesterase rather than inhibition of prostaglandin synthesis. Newcombe *et al.* (1974) have previously demonstrated phosphodiesterase inhibition by high doses of indomethacin (IC_{50}, 2.3×10^{-4}M).

In assessing the limited effect of indomethacin, the possibility of drug decomposition needs to be considered. Indomethacin rapidly decomposes in highly alkaline (pH 10 to 11) solutions, and the rate constant for decomposition triples as temperature is increased from 20° to 41°C., (Hajratwala & Dawson, 1977; Curry *et al.*, 1982). However, at lower values of pH (< 9) and room temperature, indomethacin is relatively stable in aqueous solution. Curry *et al.* (1982) cite a reported half-life for indomethacin decomposition of 20 h at pH 9 and 25°C, and in their own study found no deterioration over 80 min of observation at pH 9.3 and 25°C. Therefore, in our experiments at pH 7.8 and 15°C, the fall in indomethacin concentration, if any, should not have had an appreciable influence on the experimental results. This was indeed the case, since after re-addition of freshly prepared solutions at hourly intervals for 6 h (after completion of the standard 16 h incubation), 10^{-4} M indomethacin still produced only 23% of I_{max}.

Ouabain

Ouabain causes marked relaxation of bullfrog lung, but with a clear maximal effect which was 30 to 50% of I_{max}, depending upon the size of the frog. The only difference in relaxant effect between 10^{-5} and 10^{-4} M concentrations was in the longer time to peak effect at the lower concentration. Since 10^{-5} M eventually produced the maximal relaxant effect of ouabain and 10^{-6} M produced none, the dose-response relationship, is quite steep. Further, it encompasses the linear portion of the ouabain concentration-response curve for inhibition of sodium-potassium ATPase in the bullfrog isolated choroid plexus (Wright, 1978) and for inhibition of acid secretion in the bullfrog isolated gastric mucosa (France & Durbin, 1981). Nevertheless, it should be emphasized that the high concentrations of ouabain used to inhibit sodium-potassium ATPase may produce effects by unknown mechanisms that are unrelated to inhibition of this enzyme.

The marked suppression and retardation of calcium-induced contraction in the ouabain-treated lung suggests that ouabain is impairing either transmembrane calcium movements or the link between contraction and cytosolic calcium concentration. Such effects could readily explain ouabain-induced relaxation in this preparation and do not necessarily reflect an action on sodium-potassium ATPase.

Potassium

The ouabain-potassium interactions, as seen in the present study, are unusual in that they are co-operative rather than antagonistic. Potassium is known to reduce tissue binding of ouabain and to antagonize some of its pharmacological effects (Baker & Willis, 1970). These actions have been well documented in studies with bullfrog gastric mucosa (France & Durbin, 1981), as well as in many mammalian preparations, and were the major reasons for testing potassium responses in the ouabain-relaxed frog lung. Contrary to our expectations, 26 mM potassium produced marked further relaxation rather than restoration of resting length. The failure of potassium (and/or repeated washing) to reverse ouabain-induced relaxation could indicate either that the ouabain effect is essentially irreversible or that ouabain cannot be easily displaced from this preparation.

Potassium-induced effects in smooth muscle are complex and represent the sum of multiple actions. The contractile responses to increases in extracellular potassium usually reflect an increased uptake or release of calcium (Weiss, 1975), but the mechanisms underlying the relaxant effects of potassium are poorly understood. Since potassium-relaxed preparations are frequently employed in pharmacological studies, the mechanisms through which potassium can induce relaxation are important to many fields of research.

A relaxant response to relatively low concentrations of extracellular potassium has been observed after incubation of various smooth muscle preparations in potassium deficient solutions followed by re-addition of potassium. This effect appears to result from stimulation of sodium-potassium ATPase, and is blocked by ouabain (Webb & Bohr, 1978; Webb, *et al.*, 1981). Previous studies of potassium-induced relaxation of guinea-pig tracheal smooth muscle (Souhrada & Souhrada, 1981), and frog stomach muscle (Baysal *et al.*, 1979) presumably involve this type of action because the relaxant effect was blocked by pretreatment with ouabain. This action, however, cannot explain all of the relaxant effects of potassium, since our studies show a marked relaxant effect even in the presence of ouabain. The apparent potentiation of potassium-induced relaxation in ouabain-treated frog lungs probably reflects block of the normal contractile response to potassium, i.e. an unmasking of the full relaxant effect, and is consistent with the marked impairment of calcium-induced contraction (Figure 6)

in calcium-depleted preparations. We conclude that prostaglandin synthesis contributes very little to the high level of intrinsic tone. In contrast, ouabain, at concentrations known to inhibit sodium-potassium ATPase, substantially reduced intrinsic tone and markedly impaired contractile responses to extracellular calcium or potassium. This suggests that a ouabain-sensitive mechanism, associated with the control of calcium movement, makes a major contribution to intrinsic tone.

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References

BAKER, P.F. & WILLIS, J.S. (1970). Potassium ions and the binding of cardiac glycosides to mammalian cells. *Nature*, **226**, 521–523.

BAYSAL, F., ÖNDER, S., ÖZGÜL, M. & TOYGAR, A. (1979). The effect of potassium on frog stomach muscle. *Br. J. Pharmac.*, **66**, 303–306.

BRINK, C., GRIMAUD, C., GUILLOT, C. & OREHEK, J. (1980). The interaction between indomethacin and contractile agents on human isolated airway muscle. *Br. J. Pharmac.*, **69**, 383–388.

BURNS, J.W. & DOE, J.E. (1978). A comparison of drug-induced responses on rat tracheal, bronchial and lung strip *in vitro* preparations. *Br. J. Pharmac.*, **64**, 71–74.

CASTILLO, J.C. & DE BEER, E.J. (1947). The tracheal chain. I. A preparation for the study of antispasmodics with particular reference to bronchodilator drugs. *J. Pharmac. exp. Ther.*, **90**, 104–109.

CONWAY, E.J. (1957). Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. *Physiol. Rev.*, **37**, 84–132.

CURRY, S.H., BROWN, EA., KUCK, H. & CASSIN, S. (1982). preparation and stability of indomethacin solutions. *Can. J. Physiol. Pharmac.*, **60**, 988–992.

DOWNES, H. & TAYLOR, S.M. (1982). A potent, structurally specific stimulant action of procaine in bullfrog lung. *Comp. Biochem. Physiol.*, **72C**, 39–43.

DOWNES, H. & TAYLOR, S.M. (1983). Distinctive pharmacological profile of a nonadrenergic inhibitory system in bullfrog lung. *Br. J. Pharmac.*, **78**, 339–351.

DOWNES, H., WAYSON, K.A., EDNEY, S.M. & GERBER, N. (1975). Influence of pH on the contractor effect of a convulsant barbiturate on frog lung. *Arch. int. Pharmacodyn. Thér.*, **218**, 66–76.

FARMER, J.B., FARRAR, D.G. & WILSON, J. (1972). The effect of indomethacin on the tracheal smooth muscle of the guinea-pig. *Br. J. Pharmac.*, **46**, 536P–537P.

FARMER, J.B., FARRAR, D.G. & WILSON, J. (1974). Antagonism of tone and prostaglandin-mediated responses in a tracheal preparation by indomethacin and SC-19220. *Br. J. Pharmac.*, **52**, 559–565.

FENN, W.O. (1936). Electrolytes in muscle. *Physiol. Rev.*, **16**, 450–487.

FOSTER, R.W. (1960). The paired tracheal chain preparation. *J. Pharm. Pharmac.*, **12**, 189–191.

FRANCE, V.M. & DURBIN, R.P. (1981). Ouabain binding to isolated frog gastric mucosa. *Am. J. Physiol.*, **241**, G104–G108.

GHIARA, P., PARENTE, L. & PIOMELLI, D. (1984). The cyclooxygenase pathway in the avascular heart of the frog, *Rana esculenta* L. *Gen. Pharmac.*, **15**, 309–313.

HALUSHKA, P.V., LEVANHO, A. & AUBER, M. (1980). Prostacyclin stimulates short circuit current in the isolated toad *(Bufo marinus)* urinary bladder. In *Advances in Prostaglandin & Thomboxane Research* Vol. 8, ed. Samuelsson, B., Ramwell, P.W. & Paoletti, R. pp. 1269–1272. New York: Raven Press.

HAJRATWALA, B.R. & DAWSON, J.E. (1977). Kinetics of indomethacin degradation I: Presence of alkali. *J. Pharmac. Sci.*, **66**, 27–29.

HAWKINS, D.F. & SCHILD, H.O. (1951). The action of drugs on isolated human bronchial chains. *Br. J. Pharmac.*, **6**, 682–690.

HOWELL, B.J., BAUMGARDNER, F.W., BONDI, K. & RAHN, H. (1970). Acid-base balance in cold-blooded vertebrates as a function of body temperature. *Am. J. Physiol.*, **218**, 600–606.

HUTÁS, I., HADHÁZY, P., DEBRECZENI, L. & VIZI, E.S. (1981). Relaxation of human isolated bronchial smooth muscle. *Lung*, **159**, 153–161.

JAMIESON, D. (1962). A method for the quantitative estimation of drugs on the isolated intact trachea. *Br. J. Pharmac.*, **19**, 286–294.

KRELL, R.D. (1978). Pharmacologic characterization of isolated canine bronchial smooth muscle. *Eur. J. Pharmac.*, **49**, 151–155.

MAIN, I.H.M. (1964). The inhibitory actions of prostaglandins on respiratory smooth muscle. *Br. J. Pharmac.*, **22**, 511–519.

MATHÉ, A.A., ÅSTRÖM, A. & PERSSON, N.-Å. (1971). Some bronchoconstricting and bronchodilating responses of human isolated bronchi: evidence for the existence of α-adrenoceptors. *J. Pharm. Pharmac.*, **23**, 905–910.

NEWCOMBE, D.S., THANASSI, N.M. & CIOSEK, C.P. Jr. (1974). Cartilage cyclic nucleotide phosphodiesterase: Inhibition by anti-inflammatory agents. *Life Sci.*, **14**, 505–519.

ONO, T., OHTSUKA, M., SAKAI, S., OHNO, S. & KUMADA, S. (1977). Relaxant effect of aspirin-like drugs on isolated guinea pig treacheal chain. *Jap. J. Pharmac.*, **27**, 889–898.

OREHEK, J., DOUGLAS, J.S. & BOUHUYS, A. (1975). Contractile responses of the guinea-pig trachea *in vitro*: modification by prostaglandin synthesis-inhibiting drugs. *J. Pharmac. exp. Ther.*, **194**, 554–564.

OREHEK, J., DOUGLAS, J.S., LEWIS, A.J. & BOUHUYS, A. (1973). Prostaglandin regulation of airway smooth muscle tone. *Nature (New Biol.)*, **245**, 84–85.

ROSA, L.M. & McDOWALL, R.J.S. (1951). The action of the local hormones on the isolated human bronchus. *Acta allergologica*, **4**, 293–304.

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

TAYLOR, S.M. & DOWNES, H. (1982). Bronchodilator mechanisms in bullfrog lung: differences in response to isoproterenol, theophylline and papaverine. *J. Pharmac. exp. Ther.*, **223,** 359–367.

TAYLOR, S.M., PARÉ, P.D. & SCHELLENBERG, R.R. (1984). Cholinergic and nonadrenergic mechanisms in human and guinea pig airways. *J. appl. Physiol.: Respirat. Environ. Exercise Physiol.*, **56,** 958–965.

VANE, J.R. (1971). Inhibition of Prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (New Biol.)*, **231,** 232–235.

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.

WEBB, R.C., LOCKETTE, W.E., VANHOUTTE, P.M. & BOHR, D.F. (1981). Sodium potassium-adenosine triphosphatase and vasodilatation. In *Vasodilatation*, ed. Vanhoutte, P.M. & Leusen, I. pp. 319–330. New York: Raven Press.

WEISS, G.B. (1975). Stimulation with high potassium. In *Methods in Pharmacology*, Vol. 3, ed. Daniel, E.E. & Paton, D.M. pp. 339–345. New York and London: Plenum Press.

WONG, P.Y.D., BEDWANI, J.R. & CUTHBERT, A.W. (1972). Hormone action and the levels of cyclic AMP and prostaglandins in the toad bladder. *Nature (New Biol.)*, **238,** 27–31.

WRIGHT, E.M. (1978). Ion transport across the choroid plexus. In *Transport Across Multi-Membrane Systems: Membrane Transport in Biology*, Vol. 3, ed. Giebisch, G., Tosteson, D.C. & Ussing, H.H. pp. 355–377. Berlin, Heidelberg and New York: Springer-Verlag.

YAMAGUCHI, T., HITZIG, B. & COBURN, R.F. (1976). Endogenous prostaglandins and mechanical tension in canine trachealis muscle. *Am. J. Physiol.*, **230,** 1737–1743.

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