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The effect of ouabain on tension in isolated respiratory tract smooth muscle of humans and other species

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1 The Na^+, K^+-pump has been implicated in animal models of airway hyperreactivity. We examined the effects of inhibiting the Na^+, K^+-pump and Na^+, Ca^{2+}-exchange on isometric tone of isolated trachealis from humans and other species.

2 In preparations from 5 out of 9 humans, strong spontaneous contractions (36–48 h^{-1}; up to 1.8 g) developed within 25 min.

3 Ouabain (10^{-7}–10^{-5} M) caused an immediate and sustained contraction. This response was not blocked by atropine, diphenhydramine, or cimetidine.

4 Contractions were also elicited when the normal physiological solution was changed to a K^+-free solution, a procedure which inhibits the Na^+, K^+-pump, and in reduced (15 mM) Na^+ solution, which inhibits Na^+, Ca^{2+} exchange.

5 In preparations of dog and guinea-pig isolated trachea, ouabain (10^{-5} M) caused a multiphasic response; in the rabbit, ouabain was without effect. K^+-free solution was without effect in the dog preparations and produced relaxation of the guinea-pig trachea. Guinea-pig tracheae responded to a low Na^+ solution with a strong contraction.

6 Our findings indicate that: (a) human airway smooth muscle may be a spontaneously contracting muscle, at least *in vitro*, (b) a prolonged contraction to ouabain is unique for the human airway smooth muscle among the animals tested, as is the contraction in a K^+-free medium, and (c) the contractile response does not involve acetylcholine or histamine release, but may involve a Na^+, Ca^{2+}-exchange mechanism. These results suggest that the level of Na^+, K^+-pump activity could play a role in determining the degree of bronchomotor tone in humans.

Introduction

Blockade of the Na^+, K^+-pump has been shown to cause contraction of respiratory tract smooth muscle in non-human models (Dixon & Brodie, 1903; Marco *et al.*, 1968; Souhrada & Souhrada, 1982). It has been demonstrated that sensitization of guinea-pigs with antigen is associated with hyperpolarization of the smooth muscle cells of the trachealis; much of this change in resting membrane potential can be accounted for by increased activity of the Na^+, K^+-pump (Souhrada & Souhrada, 1982; 1984). This hyperpolarization occurring after sensitization was in turn associated with an increase in the contractile response of isolated tracheal strips to histamine (Souhrada & Souhrada, 1982). Because changes in activity of the Na^+, K^+-pump may have relevance to bronchoconstrictive disorders in humans, we decided to investigate the effect of inhibiting the Na^+, K^+-pump on tone of human respiratory tract smooth muscle, and compare it with the effects of inhibiting the pump on the isolated airways of dogs, rabbits and guinea-pigs. In the course of the experiments, the development of spontaneous phasic activity in human trachealis was noted and these data also are presented.

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Methods

Human trachea was obtained during autopsies within 12 h of death. The age of the subjects varied from 22 to 87. The causes of death were various, and included myocardial infarction and trauma. The trachea was

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cleaned and slit along the anterior, long axis, opposite the muscle. Strips consisting of one transverse muscle segment, between the ends of a cartilaginous ring, were prepared, and ligatures were tied to each end of the muscle. The tissues were mounted in 10 ml water-jacketed (37°C) organ baths containing modified Krebs-Henseleit (MKH) solution of the following composition (mM): NaCl 113, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 5.7, gassed with 95% O_2 + 5% CO_2. Resting load was adjusted to the optimal level of 2 g with the muscle attached to a force-displacement transducer for the measurement of isometric tension responses. The tissues were equilibrated in MKH under the resting load for 60 min. KCl (100 mM) was then added to induce a reference contraction in order to test the ability of each tissue to contract before exposure to other agents. The tissues were then washed with MKH solution three times, over a 30 min period, before other procedures were initiated. Paired tissues from each trachea were employed for control and experimental treatments.

Studies were also performed using trachea from three other animal species. The cervical trachea was removed from male (10–20 kg) mongrel dogs after killing them with an intravenous injection of sodium pentobarbitone (100 mg kg^{-1}). Muscle strips were prepared in a similar manner to those for humans. The trachea was also removed from male rabbits (3 kg; Green Meadows Rabbitry, Murrysville, PA) after intravenous sodium pentobarbitone administration (100 mg kg^{-1}); here the cartilage at the end of the strips was left intact, and the ligatures tied to the cartilage. Male guinea-pigs (English short-hair; 0.5 kg; (male-Hla: (SR (BR)); Hilltop Laboratory Animals, Scottdale, PA) were killed by cervical dislocation, and tracheal strips containing two cartilage/muscle segments were prepared, the ligatures tied to the cartilagenous ends of the strips. In each species resting load was optimized to give maximal contraction to KCl. The resting loads used were 2 g in dog tissues, and 1 g in the rabbit and guinea-pig preparations.

After the equilibration period and the addition of KCl to obtain the reference contraction, the following experiments were performed on the preparations:

(1) The effect of ouabain. Ouabain at different concentrations was added to the MKH solution.

(2) The ability of atropine, cimetidine, or diphenhydramine to influence the ouabain-effect in human tissue. These agents were added together, and each was present in a concentration of 10^{-6} M. After 15 min, ouabain (10^{-6} M) was added. Simultaneously, control tissues were exposed to ouabain (10^{-6} M) in the absence of the blocking agents.

(3) The effect of K^+-free MKH solution on muscle tone. A K^+-free solution (containing, mM: NaCl 117.8, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgSO_4$ 1.2, NaH_2CO_3 25, and glucose 5.7) was substituted for the MKH solution. These studies were performed using human, guinea-pig and dog preparations.

(4) The effect of reduced Na^+-MKH solution on muscle contraction. This solution (containing, mM: NaCl 15, KCl 5.92, $CaCl_2$ 2.49, KH_2PO_4 1.19, $MgSO_4$ 1.49, $HaHCO_3$ 15.5, glucose 11.5 and sucrose 244) was substituted for the MKH solution and responses of human and guinea-pig trachealis tested.

(5) The effect of ouabain on responses to K^+-free solution. The K^+-free solution was substituted for the MKH solution; after 55 min K^+ (30 mM) was added. In some baths ouabain (10^{-6} M for human, and 10^{-5} M for guinea-pig tissues) was added 45 min before the addition of K^+.

The number of separate experiments (n) with human trachealis was 6–9. The n for other species was 4–6 different animals. Results are expressed as mean ± s.e.mean. The results were evaluated for differences by use of Student's t test (two-tailed) for paired samples.

Results

In 5 out of 9 human preparations, spontaneous contractions (Figure 1) were present and developed within 25 min after applying the resting load. The frequency varied from 36 to 48 per h and the amplitude of each contraction was up to a maximum of 1.8 g. The causes of death and conditions of the lungs in these 5 individuals were: (1) gunshot wound to the head; lungs normal except for aspirated blood, (2) myocarditis; moderate vascular congestion and oedema of the lungs; (3) cardiomyopathy; moderate vascular conges-

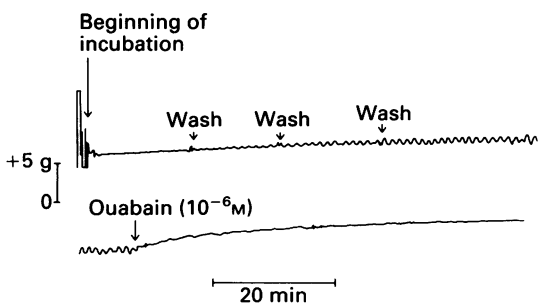

Figure 1 The effect of ouabain on resting tension in the human trachealis. Within 20 min of incubation the trachealis developed spontaneous phasic activity. Ouabain inhibited this activity and caused a sustained contraction that continued to increase after 65 min.

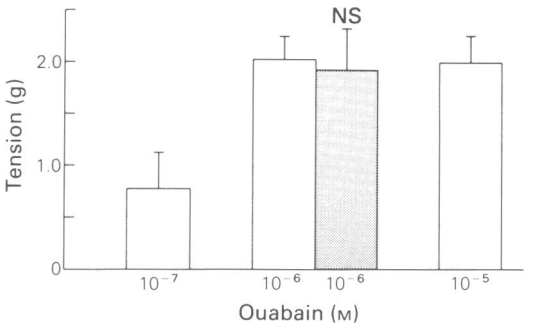

Figure 2 The effect of varying concentrations of ouabain on tension in the human trachealis (open columns) and the effect of atropine (10^{-6} M), diphenhydramine (10^{-6} M) and cimetidine (10^{-6} M) on ouabain-induced contraction (stippled column). Tension was measured 40 min after addition of ouabain. When present, the antagonists were added to the baths 15 min before ouabain. There was no significant differences in the responses to 10^{-6} M ouabain in the presence and absence of the blocking agents. Ouabain stimulated contraction in human respiratory tract smooth muscle. The effect of ouabain was not secondary to stimulation of muscarinic or histamine receptors. Each column represents the mean with vertical lines indicating s.e.mean.

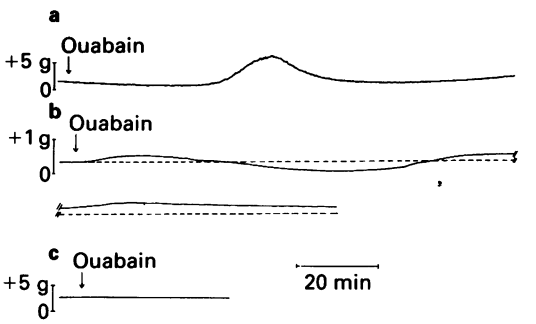

Figure 3 The effect of ouabain (10^{-5} M) on resting tension in the (a) dog, (b) English short-hair guinea-pig and (c) rabbit trachealis. In the dog and guinea-pig a transient contraction followed by relaxation occurred. In the rabbit ouabain (10^{-5} M) was without effect. In 3 out of 4 dogs there was a delay of over 30 min before an effect of the ouabain was noted.

tion of the lungs; (4) shotgun wounds to the chest and abdomen; lungs normal except in trajectory of the pellets; (5) glioblastoma of the brain; bronchopneumonia, bilateral. Similar phasic activity was not seen in the resting preparations of the guinea-pig, dog or rabbit in the 60–90 min which elapsed before the addition of experimental agents.

Ouabain at 10^{-8} M was without effect on human trachealis. At 10^{-7} M to 10^{-5} M it caused a sustained contraction, which increased in force throughout the period of observation (up to 70 min; Figures 1 and 2). When phasic activity was present, ouabain decreased its amplitude, causing it to disappear eventually.

In order to determine if the ouabain-induced contraction was secondary to stimulation of muscarinic receptors or histamine H_1- and H_2-receptors, the effect of ouabain was tested in the presence of atropine, diphenhydramine and cimetidine. These agents produced no attenuation of the ouabain-induced contraction (Figure 2).

In order to compare the effect of inhibiting the Na^+, K^+-pump with ouabain in human tissue, we determined the effect of ouabain on the guinea-pig, dog and rabbit trachealis. Ouabain (10^{-5} M) was without effect on tone in rabbit trachea but it caused characteristic responses in the guinea-pig and in the dog preparation (Figure 3). In the guinea-pig a multiphasic response occurred consisting of an initial contraction followed by a more prolonged relaxation, then contraction and relaxation. In the dog, an initial contraction was followed by relaxation to baseline and a second contraction. (Because of lack of basal tone in the dog trachealis, relaxation from baseline would not be detectable). The timing of these events is illustrated in Figure 3.

Another method of inhibiting pump activity is to decrease extracellular K^+ concentration. A K^+-free solution was substituted for the MKH solution. The human trachealis responded with a slowly-developing contraction, which in the majority of tissues was interrupted by a period of constant tone (Figure 4). The guinea-pig preparation relaxed when exposed to K^+-free solution (Figure 4; Table 1). No effect of K^+-free solution was observed on the dog trachealis.

Because contraction to ouabain may result secondarily from inhibition of Na^+, Ca^{2+}-exchange across the plasma membrane, human and guinea-pig trachealis were exposed to a reduced Na^+ MKH solution to determine if inhibition of Na^+, Ca^{2+}-exchange would cause contraction. A marked, 2-phase contraction occurred in the human and guinea-pig tissues (Figure 4, Table 1).

To investigate whether tension changes were related to alterations in electrogenic Na^+, K^+-pumping, human and guinea-pig trachealis were incubated in a K^+-free solution. K^+ (30 mM) added to this solution resulted in a relaxation of the muscle from both species, which was blocked completely by ouabain (10^{-6} M; Table 2).

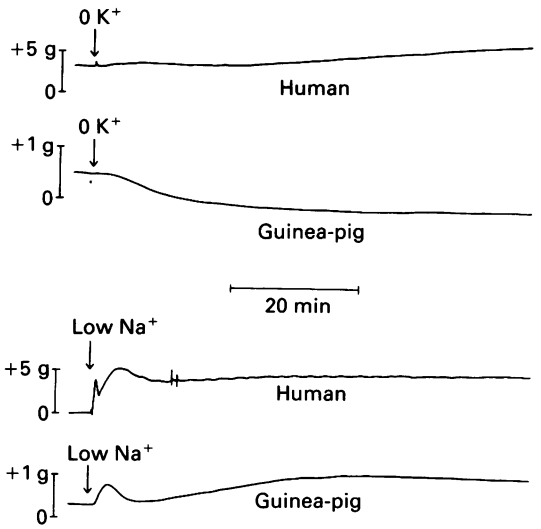

Figure 4 The effect on tension in human and guinea-pig trachealis of changing the bathing medium from Krebs-Henseleit solution (MKH) to a K^+-free MKH solution or to a reduced Na^+ (15 mM)-MKH solution. The contraction occurring in the human tissues in the absence of extracellular K^+ is consistent with inhibition of the Na^+, K^+-pump after removal of exchangeable K^+. The relaxation in the guinea-pig may be due to a predominant effect of increasing the K^+ driving force. The contraction occurring in the presence of reduced extracellular Na^+ is consistent with inhibition of a Na^+, Ca^{2+} exchange mechanism.

Table 1 The effect of altering the ionic environment on tension in the trachealis

Species	*Medium*	*Tension* (g)*
Human	K^+-free	$+0.65 \pm 0.22$
	Low Na^+	$+3.15 \pm 1.10$
Guinea-pig	K^+-free	-0.34 ± 0.10
	Low Na^+	$+0.56 \pm 0.09$

*Change in tension was measured 40 min after changing medium to K^+-free or to reduced Na^+ Krebs-Henseleit solution. + Indicates contraction, − indicates relaxation.

Table 2 The effect of ouabain on K^+ (30 mM)-induced relaxation of the trachealis in K^+-free medium

Species	*Ouabain*	*Tension* (g)*
Human	−	-0.25 ± 0.05
	+	$+1.75 \pm 0.15$†
Guinea-pig	−	-0.23 ± 0.02
	+	$+0.09 \pm 0.01$†

*Change in tension was measured 2.5 min after addition of K^+; in the last column, + indicates contraction, − indicates relaxation. † $P < 0.05$ compared with the control (ouabain absent) group.

Discussion

Spontaneous phasic activity of respiratory tract smooth muscle has been observed in a number of non-human species (see Widdicombe, 1963 for a review). *In vitro* it has rarely been seen without either prolonged incubation or pharmacological treatment. In the guinea-pig, it has been elicited after prolonged incubation of trachealis, over 5 h (Souhrada & Dickey, 1975). It has been induced in non-human species by incubation in the presence of tetraethylammonium (Stephens *et al.*, 1975), histamine (Kirkpatrick, 1975) and acetylcholine (Wick, 1952). *In vivo*, spontaneous activity of the trachealis has been demonstrated in the dog (Loo *et al.*, 1957) rabbit (Souhrada & Dickey, 1975) and guinea-pig (Souhrada & Dickey, 1975).

It has been observed in humans (Kneussl & Richardson, 1978), that trachealis removed from 3 cadavers exhibited spontaneous mechanical activity. These 3 individuals were suffering from pneumonia or septic shock; the spontaneous activity was attributed to those pathologies. In our series of experiments, 4 of the 5 patients whose trachealis showed spontaneous activity had neither infectious pneumonia nor septic shock. *In vivo* x-ray studies in humans, have yielded inconclusive evidence regarding spontaneous activity of tracheal and bronchial smooth muscle (Fleischner, 1959).

In our data the spontaneous activity when present, was observable after less than 20 min of incubation. In the other species studied, spontaneous activity was not observed during a 90 min period of incubation.

Because the human tissue was cadaveric in origin, it is conceivable that the phasic activity is artifactual. However, it is also possible that the phasic activity represents the *in vivo* status of the trachealis in the human. Spontaneous activity may occur at other levels of the tracheobronchial tree.

Whatever the status of spontaneous activity of human smooth muscle, ouabain contracts it. The contraction is prolonged, the tension continuing to increase for at least 70 min. This change in tension is qualitatively different from that noted in the other species studied. In the rabbit, ouabain was without effect, and, in the guinea-pig and dog, the response to ouabain was multiphasic, the initial contractile phase

lasting less than 20 min. The basis for this differing response in the guinea-pig and dog airways is not clear.

Another method of blocking the pump is to reduce the extracellular concentration of K^+. This manoeuvre resulted in weak contraction of the human trachealis, but in relaxation of the guinea-pig trachealis (Figure 4). In a number of the human experiments, an initial contraction was followed by partial relaxation and then contraction. The precise mechanism of this complex response is unknown, but lowering external K^+ would be expected to have at least two opposing effects on the smooth muscle: (1) by increasing the K^+ driving force, transmembrane potential would increase, which in turn may result in decreased tension and (2) by decreasing Na^+, K^+-pump activity, either membrane potential would decrease (if the pump is electrogenic) or perhaps Na^+, Ca^{2+}-exchange may decrease – resulting in an elevation in intracellular Ca^{2+} and an increase in tension. It appears that in the guinea-pig the first effect predominates. In the human, contraction predominates. The contraction-relaxation-contraction sequence in some of the preparations of human trachea may be due to alternating predominance of the opposing effects. Lowering the extracellular concentration of K^+ was without effect on dog trachealis. However, the trachealis in this species has little intrinsic tone under resting conditions, making a relaxation response difficult to detect.

In guinea-pig trachea, there may be a relationship between the ouabain-induced prolonged contraction and the contraction to reduced K^+ concentrations in the bathing fluid. In a different strain of guinea-pig (the Camm-Hartley strain), Souhrada & Souhrada (1982) demonstrated both sustained contraction to ouabain and contraction in the presence of K^+-free medium, results similar to our data for the human tissues, but not guinea-pig trachealis. One may postulate that in those tissues in which both responses occur, the maintenance of relaxation in preparations under basal tension is very dependent on activity of the Na^+, K^+-pump. It appears possible that in our experiments the depolarizing effect, after inhibition of the electrogenic pump by lowering the external K^+ concentration, is more than matched by the hyperpolarizing, relaxing effect of the increased K^+ driving force.

Is the difference in the responses of guinea-pig and human airway smooth muscle secondary to the pump acting in an electrogenic manner in the one organism and not the other? Both in the human and guinea-pig, the re-addition of K^+ after incubation of trachealis in K^+-free medium induces relaxation which is blocked by ouabain. This phenomenon may be taken as evidence that the pump in both species is at least capable of electrogenic transport (Casteels, 1984).

It has been hypothesized that in vascular smooth muscle, a Na^+, Ca^{2+}-exchange mechanism exists; ouabain, by increasing intracellular Na^+, inhibits this exchange, thus resulting in contraction (Reuter *et al.*, 1973). Supporting the possibility that this mechanism may function in the human trachealis is our finding that the trachealis contracts when incubated in a low Na^+ medium (Figure 4). The same phenomenon occurs in the guinea-pig (Kawanishi *et al.*, 1984) (Figure 4) and bovine (Bullock *et al.*, 1981) trachealis and may be a characteristic of this smooth muscle.

In conclusion, we have presented data (1) raising the possibility that human respiratory tract smooth muscle may be spontaneously active, (2) demonstrating an effect of blockade of the Na^+, K^+-pump, which suggests that the level of tone of respiratory tract smooth muscle in the resting state may be influenced by the operation of the pump, (3) showing that the contractile response to ouabain does not involve acetylcholine or histamine release, (4) demonstrating the electrogenicity of the pump and (5) raising the possibility that blockade of the pump may cause a contractile response via inhibition of Na^+, Ca^{2+}-exchange. Thus, there may exist a relationship between activity of the Na^+, K^+-pump and muscle tone in human respiratory tract smooth muscle. These studies indicate that the pump has a role in determining bronchomotor tone in humans.

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