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RELAXANT EFFECTS OF LITHIUM ON GUINEA-PIG TRACHEAL SMOOTH MUSCLE *in vitro*

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1 The effect of lithium was studied on resting tesion of guinea-pig spiral tracheal strips and their responses to carbachol and histamine *in vitro*.

2 Lithium reversibly relaxed tracheal smooth muscle in a dose-dependent manner. In addition, lithium increased the ED_{50} for carbachol 10 fold and that for histamine by over 100 fold; maximum responses for each agonist were also reduced.

3 Lithium-induced relaxation of tracheal smooth muscle was unaffected by changes in extracellular calcium concentration over the range 0–11 mM, verapamil, ouabain, procaine, propranolol or reduction in extracellular sodium concentration.

4 The ability of lithium to reduce carbachol- and histamine-induced contraction of tracheal smooth muscle was also not altered by propranolol, procaine, ouabain, verapamil or lowered extracellular sodium.

5 We conclude that lithium acts directly on tracheal smooth muscle to relax it by an as yet unknown mechanism. This may or may not be related to the ability of this agent to alter agonist-induced contraction of tracheal smooth muscle.

Introduction

It has recently been reported that administration of lithium to manic-depressive patients with asthma causes marked improvement in both disorders (Nasr & Atkins, 1977; Putnam, 1978). Whereas the mechanisms underlying the effects of lithium on the central nervous system have been the subject of much study (Ebstein, Hermoni & Belmaker, 1980), those involved in its anti-asthmatic effects have not yet been investigated. It has been suggested that the improvement in breathing associated with lithium therapy is secondary to the ability of this drug to alter the course of the manic-depressive state (Nasr & Atkins, 1977). However, it is possible that lithium has a direct action on airway smooth muscle to relax it, thereby dilating the airways and increasing airflow to the lungs. In support of this possibility are reports that lithium decreases the responsiveness of smooth muscle in uterus and seminal vesicle to noradrenaline and angiotensin (Freer & Smith, 1976; 1979; Patel, Shah, Patel & Gulati, 1979). We thought it important, therefore, to study effects of lithium on resting tension of airway smooth muscle and on its responses to carbachol and histamine, two potent bronchoconstrictor agents.

Methods

Adult male guinea-pigs (350–400 g body wt.) were killed by concussion, care being taken to avoid damage to the neck and trachea. The trachea from the larynx to the bronchi was immediately removed and transferred to a dissecting dish containing Krebs solution at room temperature. The composition of the Krebs solution used throughout these experiments was (mM): NaCl 118, KCl 4.3, $MgCl_2$ 0.5, $CaCl_2$ 1.9, NaH_2PO_4 1.0, $NaHCO_3$ 25 and glucose, 11. The trachea was separated from the bronchial tree and cut spirally. The spiral tracheal strip was then transferred to a 10 ml glass organ bath containing Krebs solution maintained at 37°C, pH 7.4 and equilibrated for 60 min before the initial tension was adjusted to 1–1.3 g. Contraction of the trachealis muscle was measured with a force-displacement transducer (Grass model FT 03C) and recorded on a Grass polygraph. All drugs were added directly to the organ bath, and the concentrations were adjusted so that all volumes were less than 0.5 ml. Lithium chloride was used in all experiments. Doses of all drugs refer to the salt.

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Results

We first compared the effects of lithium with those of noradrenaline on both resting tension and carbachol-contracted trachealis muscle. Lithium chloride, in concentrations beginning at about 2 mM, caused an immediate decrease in resting tension of tracheal strips, the relaxation being dose-dependent (Figure 1). The amount of relaxation produced by Li was reproducible at any given dose (Figure 1b), and the trachea remained relaxed for as long as Li was in the bath (up to 30 min). Tension returned to control levels within 1–2 min of removal of Li from the bath. In contrast, noradrenaline did not relax resting trachealis muscle up to a concentration of 1.0 M, the highest concentration tested. However, when the trachealis muscle was contracted by carbachol, noradrenaline was considerably more powerful than lithium. Thus, the concentration of noradrenaline needed to relax carbachol-contracted trachealis muscle was 2.5×10^{-3} mM and that of lithium was 10 mM.

Beginning at a concentration of 10 mM, lithium caused a shift downward and to the right in the dose-response curves of both carbachol and histamine (Figures 2 and 3). Although in both cases, lithium increased the ED_{50} and decreased the maximum response, lithium was more effective against histamine than carbachol. Thus, against carbachol, 20 mM Li caused a 10 fold increase in the ED_{50} and decreased the maximum response to less than 80% of control. In contrast, this concentration of Li increased the ED_{50} for histamine by over 100 fold and decreased the maximum response to less than 50% of control. All responses to carbachol and histamine returned to control values when tested after the removal of lithium from the bath.

One possible mechanism for the relaxant effect of lithium is by prevention of or interference with calcium mobility. We, therefore, examined effects of varying the Ca concentration from 0 to 11 mM in the bathing medium on the direct relaxation produced by lithium. Responses of the isolated spiral tracheal strip were unaffected by changes in extracellular Ca. In another set of experiments we tested effects of ver-

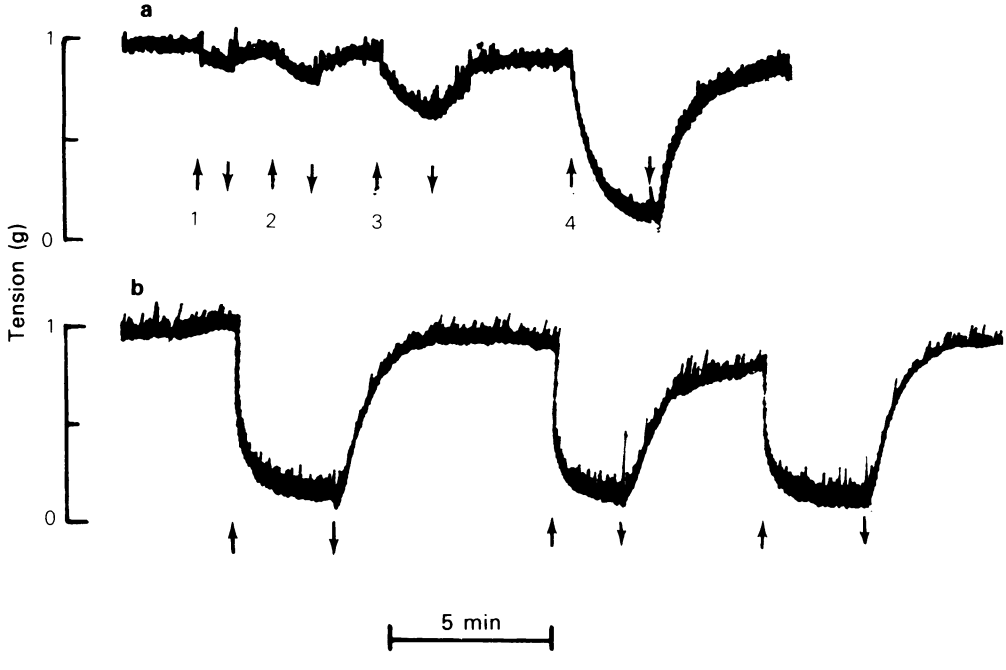

Figure 1 Relaxant effects of Li on isolated trachealis muscle. Effects of Li were determined on resting tension, which was set initially at about 1–1.3 g. (a) Relaxation in response to increasing doses of Li (1 = 2.4 mM; 2 = 11.8 mM; 3 = 23.5 mM; 4 = 59 mM). Note the immediate onset of both the response after addition of Li to the bath (upward arrow) and the return to control on washing out (downward arrow). (b) Responses to 3 successive administrations of the same dose of Li (final concentration = 59 mM). Li was added at the first arrow and washed out at the second inverted arrow.

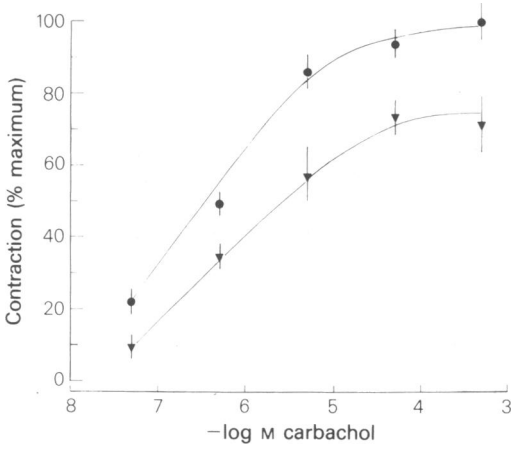

Figure 2 Effects of Li on dose-response curves of isolated trachealis muscle to contractile effects of carbachol. Each point represents the mean of 15 to 20 values from 8 to 10 experiments. Vertical lines show s.e.mean. (●) Control; (▼) Li 23.5 mM.

apamil, which reportedly blocks Ca mobility within the cell. This substance was also without effect on Li-induced relaxation; on the other hand, stimulant effects of carbachol were reduced by verapamil in a dose-dependent manner.

Lithium might also cause relaxation by release of neurotransmitter from nor-adrenergic or other inhibitory nerves in the trachea. To test this possibility, we examined effects of procaine and propranolol on lithium's ability to relax trachealis muscle; both substances were without effect.

Another possible mechanism to be considered is

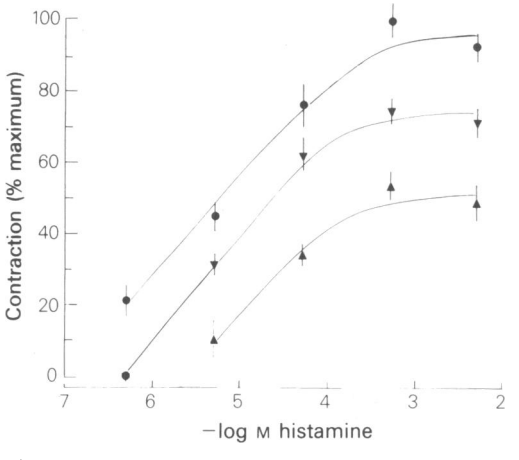

Figure 3 Effects of Li on dose-response curves of isolated trachealis muscle to contractile effects of histamine. Points as in Figure 2. (●) Control; (▼) Li 11.8 mM; (▲) Li 23.5 mM.

that lithium may activate an inhibitory process, such as a Ca-pump. Prior incubation of trachealis muscle with ouabain 10^{-4} M, for up to 1 h had no effect on lithium-induced relaxation. However, carbachol-induced contractions were markedly reduced or abolished by ouabain, as were contractile responses to transmural electrical stimulation.

Discussion

The results clearly demonstrate that lithium has a direct effect on airway smooth muscle causing relaxation as well as interfering with the stimulant effects of carbachol and histamine. The ability of lithium to block responses of smooth muscle to various stimulants has been demonstrated in several other preparations. Freer & Smith (1976, 1979) found that lithium inhibited contractions of rat uterus in response to angiotensin and acetylcholine. Similarly, Patel *et al.* (1979) found that lithium reduced the responses of rat seminal vesicles to noradrenaline. The mechanism of this effect of lithium is as yet unclear. The former authors suggested that lithium's action may involve an effect on calcium exchange induced by smooth muscle stimulants. The latter group concluded that lithium may increase the uptake of noradrenaline, thus diminishing the amount of transmitter at the receptor site. Although our experiments were not designed to test either of these mechanisms, we may deduce that neither is applicable to lithium's antagonistic effects on carbachol and histamine-induced contraction of trachealis muscle. This effect of lithium was unaltered by either variations in external calcium concentration or the presence of verapamil, although the effects of the agonists were. The actions of carbachol and histamine are not terminated by uptake into nerves or mast cells; it might be argued that lithium enters the cells via sodium channels and, in some way, interferes with subsequent permeability changes induced by the agonists. Against this possibility are the findings that identical effects of lithium on responses to histamine and carbachol were obtained when the external sodium concentration was decreased by amounts equal to the molar concentration of lithium (up to 59 mM). Another possibility, suggested by the effect of lithium on the dose-effect curves of both carbachol and histamine, is that lithium may act as a non-competitive antagonist for occupation of receptor sites or for some cellular process involved in smooth muscle contraction (see Goodman & Gilman, 1975) rather than by altering receptor affinity. Drug-induced contraction of tracheal smooth muscle involves a variety of steps from membrane depolarization (Kirkpatrick, 1975; Coburn & Yamaguchi, 1977) to alterations in levels of cyclic nucleotides

(see Wong & Buckner, 1978). Because of the ability of lithium to relax trachealis muscle directly (see below) and to alter levels of cyclic nucleotides in other tissues, notably brain (Ebstein *et al.*, 1980), it may not be unreasonable to suggest that lithium-induced alteration in nucleotide levels may play a role in antagonizing the stimulant effect of histamine and carbachol. Additional work is needed to clarify this point.

The finding that lithium directly relaxes tracheal smooth muscle is the first observation of such an effect of this substance on any mammalian smooth muscle. Neither Freer & Smith (1976, 1979) nor Patel *et al.* (1979) mentioned a direct relaxant effect in their studies, and in separate preliminary experiments we did not find that similar or higher doses of lithium relaxed guinea-pig ileum as it does guinea-pig trachea. The fact that other bronchodilators, such as noradrenaline and isoprenaline, relax only contracted tracheal smooth muscle (Fleisch & Calkins, 1976; Hurwitz, Jenne & Avner, 1977; Krell, 1978; Spilker & Minatoya, 1978) underscores our finding of a direct effect of lithium. In the present study, even a final concentration of 1.0 M noradrenaline failed to alter the resting tension of trachealis muscle. It may be argued that the concentration (2–58 mM) of lithium required to relax resting trachealis muscle is so high that the significance of the finding, especially with regard to clinical effects in asthmatic patients, may be questioned. Certainly, comparable doses to those we used would be highly toxic in humans. Against this it may be countered that the concentrations we used were within the same range as those used to investigate lithium's effects on other smooth muscle when substituted for sodium ion in the extracellular medium. Moreover, the concentration of lithium in the plasma of patients receiving it is of the order of 1 mM (0.7–1.4 mEq/l; White, Bohart, Whipple & Boyd, 1979), which, considering differences in species and route and duration of administration, means that differences between experimental and clinical concentrations may not be so vast.

The mechanism underlying the direct relaxant effects of lithium is not clear. The reproductibility and rapid reversibility of effect on trachealis muscle indicate that lithium does not act by long-lasting alterations in basic properties, such as might be produced by inhibition of enzymes or osmotic damage. The results we obtained with verapamil and alterations in extracellular Ca-ion levels suggest that lithium-induced relaxation does not involve Ca ions. Another possibility is that lithium may initiate an active relaxation mechanism involving energy (ATP)-dependent calcium uptake. This seems unlikely because prior incubation of the trachea with ouabain for up to one hour, while abolishing contractile responses to carbachol and transmural electrical stimulation, had no effect on lithium-induced relaxation. The possibility that lithium relaxes tracheal smooth muscle by releasing noradrenaline or some other inhibitory transmitter is rendered unlikely by findings that propranolol and procaine were without effect on relaxation induced by lithium. One of the remaining possibilities is that lithium may act on tracheal smooth muscle via the cyclic nucleotide system. There is abundant evidence at sites other than smooth muscle (central nervous system) that lithium does alter levels of cyclic nucleotides (Ebstein *et al.*, 1980). In this regard, we found in preliminary experiments that lithium reduces the effects of both theophylline and isoprenaline on carbachol-contracted tracheal smooth muscle. Additional studies are being carried out to clarify this possibility. Whichever mechanism is responsible for lithium's action, it is clear that the substance produces a rapid, reversible and direct relaxation of guinea-pig isolated tracheal smooth muscle. This effect, plus the ability of lithium to antagonize both histamine- and carbachol-induced contraction of trachealis muscle, may play a role in the improvement in breathing experienced by manic-depressive patients with asthma who have received lithium.

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