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Effects of Vanadate on Responses of Guinea-pig Isolated Trachea to Spasmogens

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Abstract—The effects of vanadate on the contractility of the guinea-pig isolated trachea was examined. Vanadate (0·1 mM) produced a sustained contraction that was abolished in Ca^{2+}-free EGTA (0·1 mM)-containing physiological salt solution but was resistant to verapamil (1 μM). Vanadate (0·1 mM) depressed tracheal responses to $CaCl_2$ (in Ca^{2+}-free depolarizing solution), KCl, acetylcholine, histamine and 5-hydroxytryptamine. For vanadate (10 μM), the inhibition of spasmogenic responses only reached statistical significance for histamine and 5-hydroxytryptamine. Caffeine (1 mM)-induced spasm (trachea at 20°C in the presence of indomethacin (2·8 μM)) was not affected by vanadate (10 μM–0·1 mM). Vanadate (0·1 mM) slightly depressed the responses to KCl (50 mM), acetylcholine (1 mM), histamine (1 mM) or 5-hydroxytryptamine (0·1 mM) observed in Ca^{2+}-free EGTA (0·1 mM)-containing physiological salt solution. Vanadate (0·5 mM) depressed Ca^{2+} (20 μM)-induced contraction of trachea which had been chemically skinned of its plasmalemmal membranes. The mechanism of the inhibitory effect of vanadate on tracheal responses to a variety of spasmogens remains obscure, but, under in-vitro conditions, vanadate clearly does not induce hyper-reactivity of airway smooth muscle to spasmogens.

Occupational inhalation of airborne vanadium compounds, particularly those containing vanadium in its pentavalent form (vanadate), causes bronchial asthma (US Department of Health, Education and Welfare Report 1977; Musk & Tees 1982). In the anaesthetized guinea-pig, vanadate produces bronchoconstriction and a non-specific hyper-reactivity of the airways (Nayler & Mitchell 1987). It has been suggested from clinical (Musk & Tees 1982) and animal (Nayler & Mitchell 1987) studies that the pulmonary effects of vanadate involve a direct action on the smooth muscle of airways. Other actions of an immunologic (Zenz et al 1962) or a neurogenic (Nayler & Mitchell 1987) nature may also have a role. Vanadate contracts tracheal smooth muscle isolated from the guinea-pig (Nayler & Sparrow 1983), and monkey and rabbit (Ueda et al 1985). Vanadate has a variety of actions in-vitro but the principal mechanism of vanadate-induced contraction of guinea-pig trachea appears to be intracellular Ca^{2+} mobilization linked to inhibition of Ca^{2+}-ATPase activity (Nayler & Sparrow 1983). Although the direct effects of vanadate in the guinea-pig airways have been extensively described (Nayler & Sparrow 1983; Nayler & Mitchell 1987), its effects on the responses to spasmogens have only been explored in-vivo (Nayler & Mitchell 1987).

In the present study we have examined the influence of vanadate on responses of the guinea-pig isolated trachea to a variety of spasmogens in order to determine whether vanadate is capable of producing non-specific hyper-reactivity in-vitro.

Materials and Methods

Preparation of tracheal strips

Guinea-pigs of either sex, 350–550 g, were killed by stunning and bleeding. Trachea were excised, cleaned of adhering tissue, and cut into rings of approximately 3 mm width. Rings were then opened by cutting through the cartilage and the resulting tracheal strips suspended in tissue baths containing a physiological salt solution (PSS, composition in mM: NaCl 118·4, KCl 4·7, $NaHCO_3$ 25·0, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 0·6, glucose 11·1) maintained at 37°C and gassed with 5% CO_2 in oxygen (pH 7·4). During the preparation of the tracheal strips, great care was taken not to damage the epithelium. Isometric recording of tension changes was performed using force transducers (UF-1) in conjunction with preamplifiers (Panlab Amplifier 40154) and a polygraph (Omniscribe D5000 Recorder).

The tracheal strips were subjected to an imposed tension of 1 g (Ortiz et al 1991) and a 60 min equilibration period (with changes of the PSS at 15 min intervals) was allowed before pharmacological experimentation commenced.

Assessment of the effect of verapamil or Ca^{2+}-free solution on the effect of vanadate

The effects of vanadate (0·1 mM) were assessed in paired tracheal strips bathed in standard PSS (Ca^{2+} 2·5 mM), PSS containing verapamil 1 μM, or a Ca^{2+}-free PSS containing EGTA (0·1 mM). The tissues were equilibrated with these solutions for 30 min before administration of vanadate (0·1 mM for 30 min).

Assessment of the effects of vanadate on tracheal responses to spasmogens

Two successive, cumulative concentration-effect curves were constructed for $CaCl_2$ (0·1–100 mM), KCl (0·1–100 mM), acetylcholine (1 nM–10 mM), histamine (1 nM–1 mM) or 5-hydroxytryptamine (1 nM–0·1 mM). The experiments in which $CaCl_2$ was used as the spasmogen were carried out using a K^+-rich (55 mM KCl substituted for an equimolar concentration of NaCl), Ca^{2+}-free Tris-buffered PSS as described previously (Sarriá et al 1989). The experiments

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with the other spasmogens were carried out using standard PSS.

After the initial concentration-effect curve (first curves) for one of these spasmogens had been constructed, the tissues were allocated randomly in equal numbers to test- or time-matched control groups and a second concentration-effect curve was constructed. Second curves in test tissues were constructed in the presence of vanadate (10 μM or 0·1 mM). The antagonist was present in the PSS bathing the test tissues for 30 min before and during the construction of the second concentration-effect curve.

Assessment of the effects of vanadate on responses to spasmogens observed in a Ca^{2+}-free solution

Two successive challenges with maximally-effective concentrations of KCl (50 mM), acetylcholine (1 mM), histamine (1 mM) or 5-hydroxytryptamine (0·1 mM) were carried out. The first challenge occurred while tissues were bathed in PSS (Ca^{2+} 2·5 mM) maintained at 37°C. Second challenges occurred while tissues were bathed in a Ca^{2+}-free EGTA (0·1 mM)-containing PSS in the absence (time-matched control) or presence of vanadate (0·1 mM). Tissues were exposed to the Ca^{2+}-free PSS for 30 min before and during the second challenge. The contractile responses to the second challenge with spasmogen were expressed as a proportion (%) of that observed in response to the first challenge.

Assessment of the effects of vanadate on the caffeine-induced contraction

Guinea-pig trachealis contracts in response to caffeine when the tissue is treated with a cyclo-oxygenase inhibitor and maintained at low temperature (Small et al 1988). The experimental protocol was as follows. After equilibration in PSS at 37°C containing indomethacin (2·8 μM), the bath temperature was lowered to 20°C. The tissue was then challenged twice with caffeine (1 mM), with a 40 min interval separating the two challenges. For test tissues, vanadate (10 μM or 0·1 mM) was present for 30 min before and throughout the second challenge with caffeine. Control tissues were not exposed to vanadate but were otherwise treated similarly. The contractile response to the second caffeine challenge was expressed as a percentage of the response to the first challenge.

Assessment of the effects of vanadate on skinned tracheal preparations

Segments of trachea were skinned of their plasma membranes as reported previously (Cortijo et al 1987). Tissue segments were incubated (4 h at 4°C) in a 1% (v/v) Triton X-100 solution which contained (mM): KCl 50, sucrose 150, EGTA 5, imidazole 20 and dithioerythritol 0·5 (pH 7·4). After the tissues had been rinsed for 15 min in a solution of the same composition but without Triton X-100, they were stored in a solution of (mM): EGTA 4, $MgCl_2$ 10, ATP 7·5, NaN_3 1, imidazole 20, dithioerythritol 0·5 and 50% glycerol (pH 6·7) at −20°C for up to 7 days. The segments of trachea were set up under an imposed tension of 0·5 g in 5 mL of relaxing solution containing (mM): EDTA 4, $MgCl_2$ 10, ATP 7·5, KH_2PO_4 6, NaN_3 1 and imidazole 20. This solution was maintained at 20°C and adjusted to pH 6·7 with KOH. The relaxing solution did not contain calmodulin. The tissues were allowed to equilibrate in this medium for 20 min before Ca^{2+} was added. For the relaxing solution used in these experiments at 20°C and pH 6·7, the apparent binding constant of EGTA for Ca^{2+} is 1·2 μM (Sparrow et al 1984). This value was used to calculate the amount of $CaCl_2$ to be added to the relaxing solution to achieve a given concentration of free Ca^{2+}. Three successive Ca^{2+} (1 μM) challenges were carried out in test tissues. Vanadate (0·1 or 0·5 mM) was present for 30 min before and throughout the second challenge. Thereafter vanadate was removed from the relaxing solution. Acetylcholine (0·1 mM) was added to the tissue once full relaxation had been obtained after the third Ca^{2+} challenge. Control tissues were treated in a similar way except that they were not exposed to vanadate. The response to the second and third challenge was expressed as a percentage of the response to the first challenge.

Analysis of results

Responses to spasmogens were measured from the initial baseline. When a particular pharmacological intervention resulted in a tension change which was not fully dissipated at the end of the preincubation period, the tension was reset manually to the initial value before retesting the effects of a spasmogen. The maximal effect (E_{max}) of a spasmogen was expressed as mg of force developed per mg of the tissue dry weight (24 h at 64°C). The effective concentration 50% (EC50) was estimated from each concentration-effect curve and transformed into -log values for statistical purposes. E_{max} and $-\log$ EC50 were considered respectively as estimates of efficacy and potency of the spasmogens, or alternatively, of the responsiveness and sensitivity of the preparation.

Drugs and solutions

Acetylcholine chloride, adenosine-5′-triphosphate (ATP, disodium salt), caffeine, dithioerythritol, ethyleneglycol-bis-(β-amino-ethyl-ether)-*N*-*N*′-tetraacetic acid (EGTA), histamine hydrochloride and sodium metavanadate were purchased from Sigma Chemical Co. (St Louis, MO, USA). Verapamil hydrochloride was obtained from Biosedra-Knoll (Madrid, Spain). Other chemicals used were of analytical grade (E. Merck, Darmstadt, Germany; Panreac, Barcelona, Spain). The substances were dissolved in PSS just before use. Vehicle controls (drug solvent only) were run in parallel; no significant vehicle effects were observed. With the exception of KCl (where the stated concentration was that in excess of KCl provided by the PSS) drug concentrations are expressed as final bath concentrations of the active species.

Statistics

Data are presented as means ± s.e.m. Statistical analysis of the results was performed by analysis of variance followed by Duncan's tests (Duncan 1955). Differences were considered significant when $P < 0.05$.

Results

Effect of verapamil or a Ca^{2+}-free solution on the effect of vanadate

Vanadate (0·1 mM) produced a contraction of tracheal strips reaching a maximum of 37 ± 6 mg mg^{-1} (n = 8) in 8–15 min. The contraction was sustained during the 30 min exposure to

Table 1. The effect of vanadate on the responses to $CaCl_2$, KCl, acetylcholine (ACh), histamine (HA) and 5-hydroxytryptamine (5-HT) in the guinea-pig trachea. Responses to $CaCl_2$ were obtained in a Ca^{2+}-free, depolarizing (K^+ 55 mM) solution and those to other spasmogens in PSS. Two successive concentration-effect curves were obtained. Initial curves (controls) are not shown. Second curves were obtained in the absence (time matched controls) or presence of the interventions mentioned.

	$CaCl_2$		KCl		ACh		HA		5-HT	
	E_{max}[a]	−log EC50	E_{max}	−log EC50	E_{max}	−log EC50	E_{max}	−log EC50	E_{max}	−log EC50
Time matched control	152±16	2·16±0·11	240±22	2·85±0·02	341±26	4·43±0·05	398±16	5·25±0·11	236±28	6·00±0·21
Vanadate (10 μM)	127±15	2·13±0·15	220±17	2·53±0·19	292±21	4·88±0·13	250±7[b]	5·21±0·19	142±21[b]	5·85±0·04
Vanadate (0·1 mM)	76±10[b]	2·10±0·04	115±23[b]	2·80±0·05	236±13[b]	4·71±0·20	139±8[bc]	4·81±0·10[b]	133±12[b]	6·03±0·13

[a] Maximal effect in mg force per mg of tissue dry weight. Data are means±s.e.m. of 6–8 experiments for control and test tissues. [b] $P<0.05$ compared with control. [c] $P<0.05$ compared with vanadate (10 μM).

vanadate. In tissue pretreated with verapamil (1 μM), vanadate (0·1 mM) evoked a contraction whose maximum (33±9 mg mg^{-1}; n=5) was not significantly different from that observed in paired, control tissues. In contrast, when vanadate (0·1 mM) was added to tracheal strips bathed in Ca^{2+}-free, EGTA (0·1 mM)-containing PSS a small relaxation was observed (maximum of 18±3 mg mg^{-1}) instead of the contraction seen in paired control tissues.

Effect of vanadate on tracheal responses to spasmogens

Vanadate (0·1 mM) depressed maximal responses to $CaCl_2$, KCl, acetylcholine, histamine and 5-hydroxytryptamine whereas, with the exception of histamine, the sensitivity of tissues to these spasmogens was not reduced (Table 1). Vanadate therefore depressed the concentration-effect curves to each of the spasmogens as shown in Fig. 1. Vanadate (10 μM) tended to depress maximal responses to the spasmogens tested but statistical significance was only reached for histamine and 5-hydroxytryptamine (Table 1).

Effect of vanadate on the responses to challenge with spasmogens in a Ca^{2+}-free solution

Two consecutive challenges with KCl (50 mM), acetylcholine (1 mM), histamine (1 mM) or 5-hydroxytryptamine (0·1 mM) were obtained, the first in PSS (Ca^{2+} 2·5 mM), and the second in a Ca^{2+}-free EGTA (0·1 mM)-containing PSS. In control tissues (absence of vanadate), the response to the second challenge was only depressed for KCl and 5-hydroxytryptamine. Responses to histamine tended to be also inhibited but the difference failed to reach statistical significance. Responses to acetylcholine were not affected. In test tissues, vanadate (0·1 mM) slightly depressed contractions to all the spasmogens tested (Fig. 2) when compared with the equivalent responses observed in the time-matched control tissues.

Effect of vanadate on the caffeine-induced contraction

Two consecutive challenges with caffeine (1 mM) at 20°C in PSS containing indomethacin (2·8 μM) were carried out. Responses to the second challenge in control tissues (absence of vanadate) did not differ (99±7%, n=6) from those of the first challenge (100%). In test tissues, vanadate (10 μM or 0·1 mM) did not affect responses to the second caffeine challenge (102±11% and 95±4% of the response to the initial challenge, respectively; n=6).

Experiments in skinned trachea

Skinned tracheae were challenged three successive times with Ca^{2+} (20 μM). Control experiments showed that the contrac-

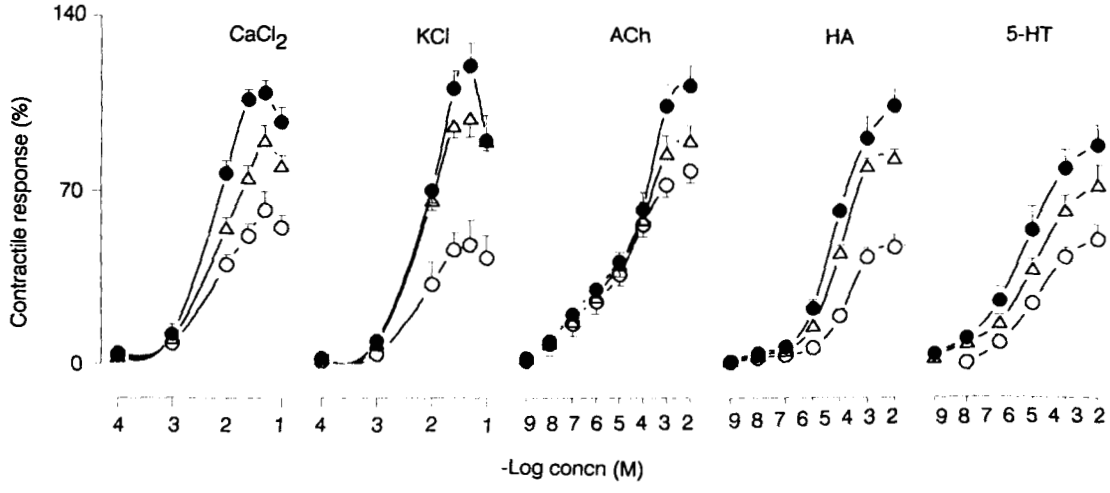

FIG. 1. Log concentration-effect curves for $CaCl_2$ (in a Ca^{2+}-free, K^+-depolarizing solution), KCl, acetylcholine (ACh), histamine (HA) and 5-hydroxytryptamine (5-HT) in guinea-pig isolated trachea. Two successive concentration-effect curves were generated. Initial curves are not shown. Second curves were constructed in the absence (time-matched controls ●) or presence of vanadate (10 μM △ or 0·1 mM ○). Results are presented as percentage of maximal responses obtained in the initial curves and are means of 6–8 experiments; s.e.m. is shown by vertical bars.

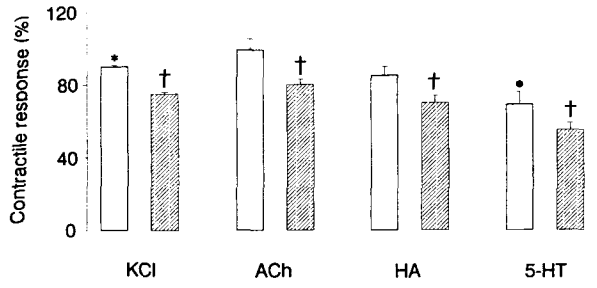

FIG. 2. Contractile responses of the guinea-pig trachea to KCl (50 mM), acetylcholine (ACh, 1 mM), histamine (HA, 1 mM) or 5-hydroxytryptamine (5-HT, 0·1 mM). Two successive challenges were generated. First challenges in PSS (Ca^{2+} 2·5 mM) are not shown. Second challenges were constructed in a Ca^{2+}-free EGTA (0·1 mM) solution in the absence (time-matched controls □) or presence of vanadate (0·1 mM ■). Responses are presented as percentage of that in the first challenge. Column heights indicate the mean of 6 experiments and vertical lines indicate the s.e.m. $*P < 0.05$ compared with the first challenge in PSS; $\dagger P < 0.05$ compared with the second challenge in time-matched control tissues.

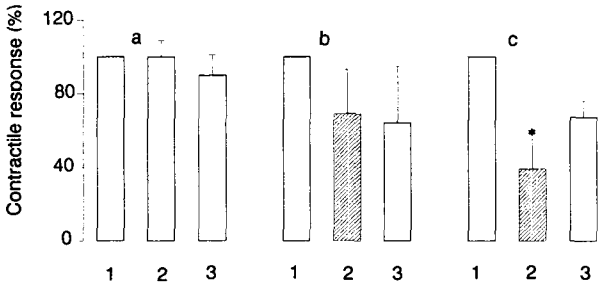

FIG. 3. The effect of vanadate on the responses of skinned trachea to Ca^{2+}. The abscissae indicate the first, second and third (1, 2, 3) challenge with 20 μM Ca^{2+}. The ordinates indicate the response as a percentage of the response to the first Ca^{2+} challenge. Panel a shows responses in control tissues. Panels b and c show responses in test tissues where the shaded columns indicate the presence of vanadate 0·1 mM (b) or 0·5 mM (c). Column heights represent the mean value of 5–6 experiments and vertical lines indicate the s.e.m.. $*P < 0.05$ compared with time-matched control.

tile response was similar in the three challenges (Fig. 3a). When test tissues were compared with their appropriate time-matched controls, it was observed that vanadate (0·1 mM) tended to depress Ca^{2+}-induced contraction but the effect did not reach statistical significance (Fig. 3b). Vanadate (0·5 mM) significantly depressed Ca^{2+}-induced contraction (Fig. 3c). Acetylcholine (100 μM) failed to contract the skinned preparations.

Discussion

Vanadate (0·1 mM) produced a sustained contraction of guinea-pig isolated trachea. This contraction has been previously demonstrated to represent a direct response, i.e. not secondary to neurotransmitter or mediator release (Nayler & Sparrow 1983). Guinea-pig tracheal contraction to vanadate was abolished in Ca^{2+}-free EGTA (0·1 mM)-containing PSS but it was resistant to verapamil (1 μM), a concentration which markedly suppressed KCl-induced tracheal spasm (Foster et al 1984). These results confirm those of Nayler & Sparrow (1983) and Ueda et al (1985), and support the view that the mechanism underlying vanadate-induced contractions of airway smooth muscle is not equivalent to the mechanism of vanadate-induced contraction in other smooth muscles. For example, facilitation of Ca^{2+} entry through voltage-dependent Ca^{2+} channels appears to be responsible for the stimulant effect of vanadate on mechanical activity in the rat portal vein (Shimamura & Sunano 1988).

The dependence of vanadate (0·1 mM)-induced contraction on extracellular Ca^{2+} ideally should be compared with that observed with other spasmogens under the same experimental conditions. Exposure to a Ca^{2+}-free EGTA (0·1 mM)-containing PSS slightly depressed KCl (50 mM)- and 5-hydroxytryptamine (0·1 mM)-induced responses, and tended to depress responses to histamine (1 mM) although this failed to reach statistical significance. Exposure to Ca^{2+}-free, EGTA (0·1 mM)-containing PSS did not affect acetylcholine (1 mM)-induced contraction. EGTA (0·1 mM) in the Ca^{2+}-free solution chelates extracellular Ca^{2+} and Ca^{2+} bound to superficial binding sites on the sarcolemma. Intracellular stores of Ca^{2+} are not readily affected by EGTA and the maintenance of responses to spasmogens like acetylcholine indicates that intracellular stores are intact. These data suggest that contraction produced by vanadate (0·1 mM) involves predominantly mobilization and utilization of extracellular Ca^{2+} or a superficial pool of Ca^{2+} that is accessible to EGTA. The release of Ca^{2+} from a sequestered intracellular store appears not to be important although it cannot be excluded.

Vanadate (0·1 mM) may promote Ca^{2+} entry through routes not susceptible to verapamil, i.e. pathways different from voltage-dependent Ca^{2+} channels. The nature of these pathways is not known. One could be the Na^+/Ca^{2+} exchanger (Di Polo 1989) which may be stimulated following an accumulation of intracellular Na^+ due to inhibition of Na^+/K^+-ATPase (Nechay 1984). However, ouabain did not inhibit vanadate-induced contraction of guinea-pig trachea (Nayler & Sparrow 1983) and vanadate had negligible effects on sodium content in monkey and rabbit trachea (Ueda et al 1985). This suggests that inhibition of Na^+/K^+-ATPase is not involved in the contractile action of vanadate. In addition, inhibition of active extrusion of Ca^{2+} from the cell or inhibition of Ca^{2+} uptake by intracellular storage sites (i.e. sarcoplasmic reticulum) could also be responsible for the contraction to vanadate. Both re-sequestration and extrusion are postulated to be due to the activation of Ca^{2+}-ATPase (Van Breemen et al 1979) which is inhibited by vanadate (O'Neal et al 1979; Dupont & Bennet 1982). The relative contribution of each one of these mechanisms to the contractile effect of vanadate in the guinea-pig trachea remains to be determined.

Vanadate (0·1 mM) significantly depressed tracheal responses to $CaCl_2$ (in Ca^{2+}-free depolarizing solutions), KCl, acetylcholine, histamine and 5-hydroxytryptamine. This concentration of vanadate was chosen as being close to the EC50 value of vanadate as a contracting agent in the guinea-pig isolated trachea (Nayler & Sparrow 1983). With a smaller concentration of vanadate (10 μM) antagonism of spasmogenic responses only reached statistical significance for hista-

mine and 5-hydroxytryptamine. Caffeine (1 mM)-induced spasm was not affected by vanadate (10 μM–0·1 mM). When vanadate (0·1 mM) was tested in tracheal strips bathed in Ca^{2+}-free EGTA (0·1 mM) solution and challenged with KCl (50 mM), acetylcholine (1 mM), histamine (1 mM) or 5-hydroxytryptamine (0·1 mM), significant inhibition of responses to spasmogens was obtained, but the extent of the inhibition tended to be smaller than that observed in PSS. The role of Ca^{2+} in the contraction of guinea-pig trachealis to KCl, agonists acting on specific membrane receptors, and caffeine has been previously studied (Foster et al 1983, 1984; Small et al 1988; Rodger & Small 1991). The mechanism underlying this inhibitory effect of vanadate on tracheal responses to spasmogens, with the exception of caffeine, is unclear. The effects of vanadate on Na^+/H^+ exchange, Na^+/Ca^{2+} exchange, or protein kinase C activity are not known. It could be that an already elevated intracellular concentration of Ca^{2+} impeded additional Ca^{2+} entry or refilling of intracellular Ca^{2+} stores. Another possibility is that vanadate interferes with intracellular contractile machinery. Nayler & Sparrow (1983) found that vanadate ($EC50 \approx 0{\cdot}1$ mM) relaxed skinned tracheal fibres of guinea-pig, which had been previously contracted by maximal Ca^{2+} (21 μM). In the present study, vanadate (0·5 mM) significantly depressed Ca^{2+} (20 μM)-induced contraction of skinned trachea, a finding which, in spite of some methodological differences, is in excellent agreement with that of Nayler & Sparrow (1983). Although vanadate readily penetrates into the cytoplasm it is not likely that this effect on the contractile apparatus is important in the intact cell since vanadate itself contracted guinea-pig trachea in a concentration-dependent fashion up to 1 mM (Nayler & Sparrow 1983). Obviously, Ca^{2+} mobilization by vanadate overcomes the inhibitory effect at the level of the contractile apparatus.

The effect of vanadate on tracheal responses to spasmogens in-vitro has not been studied previously although Ueda et al (1985) reported that vanadate (0·5 mM) inhibits K^+ (154·1 mM)-induced contraction of monkey and rabbit tracheal smooth muscle and Ishii & Shimo (1985) found that vanadate (30 μM) augmented the contractile responses of the rat trachea to acetylcholine and KCl. In the study of Ishii & Shimo (1985) a similarity was suggested to exist between the mechanism of cooling- and vanadate-induced supersensitivity to spasmogens. However, in the guinea-pig isolated trachea, Ishii & Shimo (1986) found that cooling produced subsensitivity to a number of spasmogens but the effect of vanadate was not reported.

Nayler & Mitchell (1987) reported hyper-reactivity to histamine, acetylcholine and 5-hydroxytryptamine after exposure to vanadate in anaesthetized guinea-pigs and suggested that bronchial airway smooth muscle itself is a site where vanadate was acting to enhance reactivity. Clearly, results from the present study show that this is not the case and alternative mechanisms probably underlie vanadate-induced hyper-reactivity in-vivo.

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