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Airways hyperreactivity and bronchoconstriction induced by vanadate in the guinea-pig

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1 The characteristics of vanadate-induced bronchoconstriction and airways hyperreactivity were observed in spontaneously breathing anaesthetized guinea-pigs by measurement of airways resistance (R_{aw}) and dynamic lung compliance (C_{dyn}). Vanadate (0.3–3 mg kg^{-1} i.v. over 25 min) increased R_{aw} and decreased C_{dyn} in a reversible, dose-related manner. This action (1 mg kg^{-1} vanadate) was not inhibited by atropine (1 mg kg^{-1} i.v.), propranolol (1 mg kg^{-1} i.v.) or bilateral vagotomy, suggesting a direct effect on the airways smooth muscle.

2 An aerosol of vanadate (10% w/v in H_2O) for 3 min decreased C_{dyn} by 19.5% ($P < 0.05$, $n = 6$) but caused no change in R_{aw}.

3 Histamine (3 μg kg^{-1} i.v.) caused a bronchoconstriction which was enhanced by vanadate in a dose-related manner. This hyperreactivity (after 1 mg kg^{-1} i.v. vanadate) was unchanged after propranolol or bilateral vagotomy, but was partly blocked by atropine (enhancement by vanadate of the C_{dyn} change to histamine was diminished, $P < 0.02$, $n = 3$).

4 Bronchoconstrictor responses to acetylcholine (6 μg kg^{-1} i.v.) and 5-hydroxytryptamine (6 μg kg^{-1} i.v.) were also enhanced by vanadate (1 mg kg^{-1} i.v.). Hyperreactivity after vanadate to the three bronchoconstrictors tested continued during vanadate infusion and was reversed 45 min after cessation of infusion.

5 Histamine (3 μg kg^{-1} i.v.) caused a transient tachypnoea which was also enhanced by vanadate (0.3–3 mg kg^{-1} i.v.), in a dose-related manner, in association with the increased reactivity of the airways ($r = 0.66$, $n = 11$).

6 It is concluded that vanadate-induced airways hyperreactivity is non-vagal (efferent) and largely non-cholinergic in origin and appears to involve an action of vanadate within the lung itself.

Introduction

Bronchoconstriction and airways hyperreactivity occur after exposure to airborne vanadium compounds (U.S. Dept. of Health, Education & Welfare Report, 1977; Musk & Tees, 1982) and this is most pronounced with compounds of the pentavalent form of vanadium, vanadate. The development of these pulmonary effects can occur after a single exposure in non-atopic individuals, suggesting a direct action on the airways (Musk & Tees, 1982), although the involvement of an immunologically-based sensitivity reaction has also been suggested (Zenz *et al.*, 1962).

Intact isolated airways smooth muscle of the guinea-pig from both central and peripheral airways is contracted directly by vanadate (1–1000 μM), possibly by an action of vanadate in mobilizing intracellular Ca^{2+} (Nayler & Sparrow, 1983). It is not known whether a similar spasmogenic action underlies vanadate-induced bronchoconstriction *in vivo* in humans. Vanadate contracts other smooth muscle tissues, including monkey and rabbit trachea, (Huot *et al.*, 1979; Ozaki & Urakawa, 1980; Garcia *et al.*, 1981; Ueda *et al.*, 1985), but in intestinal smooth muscle it causes a biphasic effect; inhibition of spontaneous activity followed by enhanced contraction (Hudgins & Bond, 1981). The initial inhibitory response appears to involve the release of an inhibitory transmitter, a catecholamine, from intramural sympathetic nerve endings or enterochromaffin cells. Vanadate can also enhance smooth muscle reactivity to other spasmogens. In rats for example, vanadate administered in drinking water significantly enhanced reactivity *in vivo* to noradrenaline and angiotensin II, although no

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sustained increases in blood pressure to vanadate were noted (Jadhav & Jandhyala, 1983). Vanadate has also been shown to exert centrally mediated effects, on blood pressure and heart rate, after intracerebroventricular administration in dogs (Hom *et al.*, 1982).

The aim of this study was firstly to examine whether the bronchoconstrictor effect of vanadate *in vivo* in the guinea-pig is attributable to a direct action on airways smooth muscle or to indirect effects via nervous reflexes and, secondly, to examine the characteristics of the vanadate-induced hyperreactivity. Airways resistance (R_{aw}) and dynamic lung compliance (C_{dyn}) were used to assess changes in lung function in response to vanadate given either by an aerosol or intravenously (i.v.). The effect of vanadate on airways reactivity to i.v. histamine, acetylcholine and 5-hydroxytryptamine was also assessed. The role of autonomic reflexes in the bronchoconstriction and airways hyperreactivity induced by vanadate was assessed by bilateral vagotomy, and by atropine or propranolol pretreatment.

Methods

Guinea-pigs of either sex and weighing 300–450 g were anaesthetized with 1.5 g kg^{-1} urethane i.p. One or both jugular veins were cannulated for drug injection and a carotid artery was cannulated for recording blood pressure (Narco Bio-system P-100A pressure transducer). Rectal temperature was maintained at 38 ± 0.5°C.

Airflow and tidal volume were measured using a pneumotachograph flow head which was connected to a tracheal cannula in conjunction with an electrospirometer (Mercury Electronics, Glasgow). Intrapleural pressure was measured with a differential gas pressure transducer (Grass PT5A), one port of which was connected to a needle (14 gauge) in the intrapleural space and the other to a side arm in the tracheal cannula. Signals (flow, tidal volume and intrapleural pressure) were amplified and displayed on a Washington (BioScience Sheerness) chart recorder.

Airways resistance (R_{aw}) and dynamic lung compliance (C_{dyn}) were calculated from the airflow, tidal volume and intrapleural pressure signals by the method of Amdur & Mead (1958) on-line using a BBC microcomputer (Mitchell, 1985). Basal, i.e. control, R_{aw} and C_{dyn} were determined from the mean of not less than 4 breaths taken before drug (e.g. histamine) administration. Measurements of R_{aw} and C_{dyn} were similarly performed during the action of bronchoconstrictor drugs and drug-induced changes in R_{aw} and C_{dyn} were expressed as a percentage of this basal control value. Drugs were dissolved in 0.9% w/v saline and administered in volumes <0.5 ml by either a bolus injection (in <2 s) or by infusion at a constant rate for up to 35 min.

Artificial ventilation was used only when bilateral vagotomy (vago-sympathectomy) was performed, since respiration then became depressed and irregular. These animals were mechanically ventilated (70 breaths min^{-1}) at not <1.2 times their normal tidal volume. This maintained $P_E O_2$ (i.e. partial pressure in expired air) at normal levels (approximately 17%) although $P_E CO_2$ generally fell to <25 mmHg, determined using a Datex CO_2/O_2 monitor.

Vanadate infusion

Vanadate was usually infused intravenously at a rate of 0.015 ml min^{-1} over 25 min to give a dose of 1 mg kg^{-1} (vanadate salt). At high vanadate doses (>10 mg kg^{-1} over 25 min) increases in mean arterial blood pressure and slight depression of respiration were observed. The bronchoconstrictor effect of vanadate (up to 3 mg kg^{-1} over 25 min) was assessed from changes in R_{aw} and C_{dyn} following vanadate infusion. Changes in reactivity (see below) to response matched doses of histamine (3 μg kg^{-1}), 5-hydroxytryptamine (5-HT, 6 μg kg^{-1}) and acetylcholine (ACh, 6 μg kg^{-1}) were also investigated at the end of vanadate infusion. To determine the effect of vanadate on airways reactivity to each bronchoconstrictor drug, at least 3 responses to the drug were elicited at 20–30 min intervals before vanadate infusion and the mean % change from control R_{aw} and C_{dyn} determined. This was then compared with the change in R_{aw} and C_{dyn} evoked by single drug challenges made at various times during or after the vanadate infusion. Changes in reactivity, i.e. *reactivity ratio*, to histamine, 5-HT and ACh were calculated by dividing the (% change in R_{aw} and C_{dyn} after vanadate) by the (% change in R_{aw} and C_{dyn} before vanadate, mean value).

As well as a bronchoconstriction each drug caused a brief tachypnoea. The % increase in respiratory rate (RR) to each drug was compared before and after vanadate, the (% increase in RR to drug after vanadate) divided by the (% increase in RR to drug before vanadate) producing what was termed the tachypnoea ratio.

Atropine and propranolol pretreatment

The effect of muscarinic receptor and β-adrenoceptor blockade on vanadate-induced responses was determined using atropine (1 mg kg^{-1}, i.v. over 4 min) and propranolol (1 mg kg^{-1}, i.v. over 6 min), respectively. After atropine administration histamine responses decreased. Accordingly, the histamine challenge was increased from 3 to 6 μg kg^{-1}. Responses to the increased histamine challenge became constant 50–90 min after atropine administration. Control measurements of histamine-induced changes in R_{aw}

and C_{dyn} were performed at this time as was the test measurement which followed, made at the end of vanadate infusion (1 mg kg^{-1} over 25 min).

A similar protocol was used in experiments using propranolol except that reactivity to 3 $\mu g\,kg^{-1}$ histamine was compared before and after vanadate. Control and test responses were measured within 80 min of propranolol injection, during which time control histamine responses were constant.

Vanadate aerosol exposure

Animals were exposed to an aerosol containing vanadate (10% w/v in water) for either 1 min or 3 min by placing their heads in an enclosed perspex chamber (free gas volume of approximately 500 ml) into which the outflow from an Inspiron MiniNeb nebulizer was directed. The nebulizer output was 0.27 ml min^{-1} with a particle size of 2 μm (mass median diameter) generated using an airflow drive rate of 10 l min^{-1} (ie. vanadate = 2.7 mg l^{-1} air). For these experiments the tracheal cannula had an extra side arm which was connected to a 2-way tap system which enabled the inspired air to be switched between the room environment and the chamber containing vanadate aerosol. Recording of airways responses during exposure to aerosol was not possible since the flow head was blocked during this time to permit inspiration of the chamber contents only.

At the conclusion of exposure the tracheal cannula was aspirated to remove any fluid deposits which may have affected the measured values (of R_{aw} in particular), and R_{aw} and C_{dyn} were then determined. Histamine (3 $\mu g\,kg^{-1}$) challenge occurred 2 min after the end of aerosol exposure (ie. either 3 min or 5 min from the beginning of aerosol exposure). Experiments were also performed using a 1.6 osmol l^{-1} NaCl solution to control for the osmotic effect of the vanadate (10% w/v, 1.6 osmol l^{-1}) solution.

Drugs and chemicals

Vanadate solutions ($NaVO_3$, pH 7.3, Hopkins & Williams) were prepared daily. The following drugs (Sigma) were used; histamine diphosphate, 5-hydroxytryptamine (creatinine sulphate complex), acetylcholine chloride, atropine sulphate, propranolol hydrochloride and urethane (ethyl carbamate, Ajax).

All chemicals used were analytical grade and solutions were made with twice deionized water.

Statistics

Results are expressed as the mean ± s.e.mean with the number of experiments (n) in parentheses. Statistical evaluation was performed by means of Student's t test with $P < 0.05$ regarded as significant.

Results

Vanadate-induced bronchoconstriction

Basal values for R_{aw} and C_{dyn} were 0.20 ± 0.01 cmH_2O $ml^{-1}\,s^{-1}$ and 0.47 ± 0.03 ml cmH_2O^{-1}, respectively, in 12 spontaneously breathing guinea-pigs. An infusion of 1 mg kg^{-1} vanadate over 25 min increased R_{aw} by 9.1 ± 2.5% and decreased C_{dyn} by 13.4 ± 2.7% ($P < 0.01$, $n = 12$, Figure 1a and b). These effects were dose-related (Figure 1c) and were reversed 10–15 min after the infusion was stopped.

The bronchoconstriction produced by the 1 mg kg^{-1} vanadate infusion (evident as increases in R_{aw} and decreases in C_{dyn}) was not affected ($P > 0.1$) by atropine (R_{aw} = 8.3 ± 4.9% and C_{dyn} = 8.0 ± 3.6%, $n = 3$) or propranolol (R_{aw} = 17.7 ± 6.4% and C_{dyn} = 15.7 ± 3.0%, $n = 3$) pretreatment or bilateral vagotomy (R_{aw} = 10.0 ± 3.2% and C_{dyn} = 18.3 ± 5.1%, $n = 4$).

Exposure to an aerosol of 10% (w/v) vanadate for 1 min did not produce significant changes in R_{aw} and C_{dyn}, but after a 3 min exposure C_{dyn} was decreased by 19.5 ± 7.5% ($P < 0.05$, $n = 6$) but a negligible change in R_{aw} occurred. An osmotically equivalent saline solution produced no consistent changes in bronchial calibre in 3 animals.

Hyperreactivity to histamine, 5-hydroxytryptamine and acetylcholine after vanadate

Histamine (3 $\mu g\,kg^{-1}$) caused a short-lived increase in R_{aw} (79 ± 30%) and decrease in C_{dyn} (30 ± 8%) in 5 animals and a transient tachypnoea (increase above basal RR of 46 ± 19%). After vanadate (1 mg kg^{-1}) the % change in R_{aw} and C_{dyn} in response to histamine was greatly enhanced (Figures 2 and 3). The increase in R_{aw} and the decrease in C_{dyn} to histamine after vanadate, i.e. reactivity ratio, was now 1.95 ± 0.08 × ($P < 0.001$) and 1.79 ± 0.14 × ($P < 0.01$) the control changes, respectively ($n = 5$). The magnitude of this increased reactivity was consistent for control increases in R_{aw} in response to histamine, of up to 200% and decreases in C_{dyn} of up to 40–50%. Moreover, the extent of the hyperreactivity to histamine after vanadate infusion was dose-dependent, increasing with doses of vanadate (Table 1). After stopping the vanadate infusion the hyperreactivity to histamine decreased, and was abolished by 45 min (Figure 3), although it persisted if vanadate infusion was continued.

The enhanced reactivity after vanadate was not confined to bronchoconstrictor responses provoked by histamine. Increases in reactivity were also seen to response matched doses of ACh (6 $\mu g\,kg^{-1}$) and 5-HT (6 $\mu g\,kg^{-1}$), although the extent varied with each agent (Table 2). Maintenance of hyperreactivity during

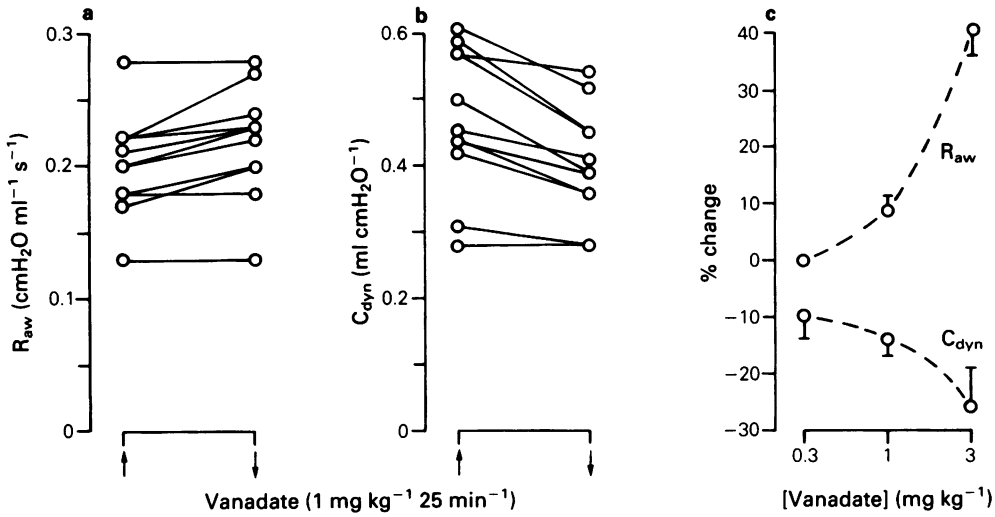

Figure 1 (a) Effect of vanadate infusion on airways resistance (R_{aw}, cmH_2O ml^{-1} s^{-1}) in 12 guinea-pigs (values for 2 animals were identical, therefore only 11 data lines). R_{aw} at the beginning of vanadate (1 mg kg^{-1} over 25 min, ↑) is compared with R_{aw} at the end of infusion (↓). (b) Changes in dynamic lung compliance (C_{dyn}, ml cmH_2O^{-1}) to vanadate in the same animals at the beginning (↑) and end (↓) of infusion. (c) Effect of vanadate on R_{aw} and C_{dyn}, as a % change from the control value at the beginning of infusion. Vanadate (0.3–3 mg kg^{-1}) was infused over 25 min and each point is the mean % change in R_{aw} and C_{dyn} from 3 or more animals; vertical lines show s.e.mean.

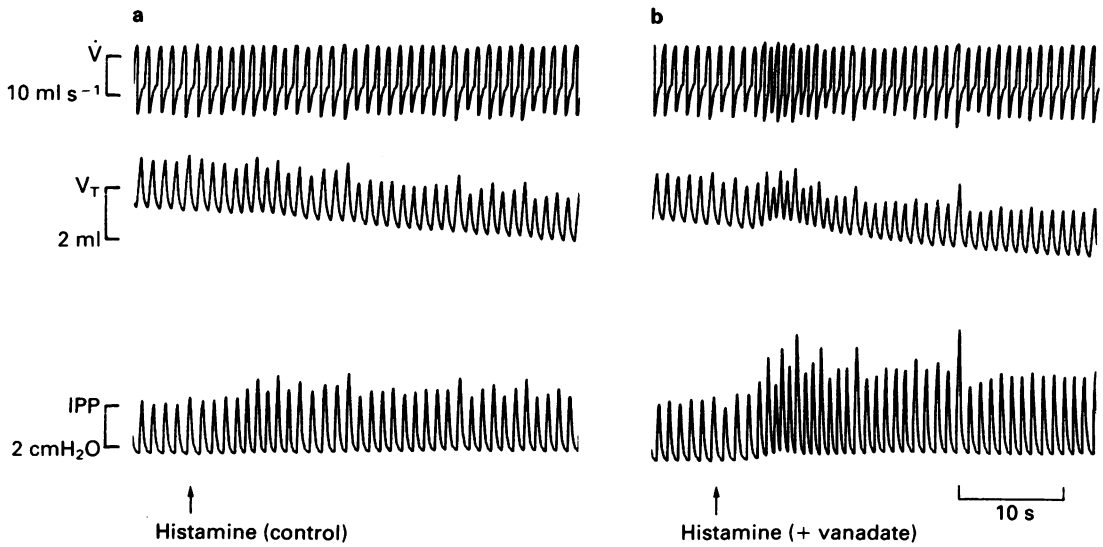

Figure 2 (a) Trace showing flow ($\dot{V}$), tidal volume (V_T) and intrapleural pressure (IPP) signals in response to a control histamine challenge (3 μg kg^{-1} i.v.). In this animal histamine increased R_{aw} by 57% and decreased C_{dyn} by 22%. Inspiration is indicated by an upward pen deflection. (b) Response to histamine in the same animal at the end of a vanadate infusion (+ vanadate, 1 mg kg^{-1} over 25 min). In this case histamine caused a 122% increase in R_{aw} and a 46% fall in C_{dyn} (i.e. reactivity ratio = 2.14 times and 2.09 times control, respectively). The drift in the V_T signal was due to drift in the integrator circuit of the electrospirometer.

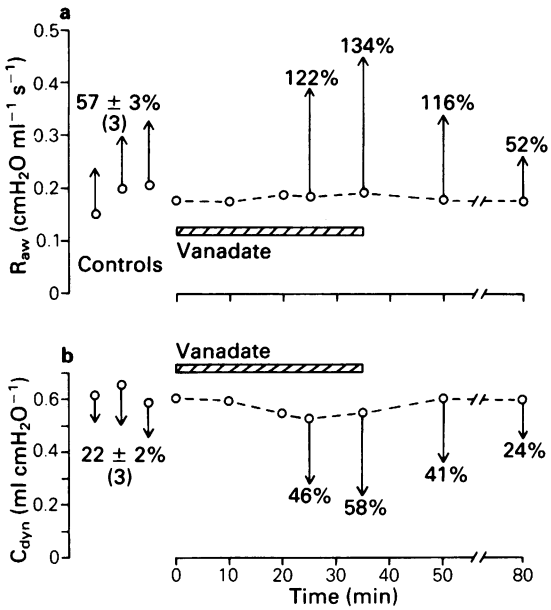

Figure 3 (a) Effect of histamine (3 μg kg^{-1}) on airways resistance (R_{aw}, cmH$_2$O ml^{-1} s^{-1}) before (control) and after vanadate infusion. The increase in R_{aw} in response to histamine challenge (↑) was expressed as a % increase above the basal R_{aw} value (O). The mean ± s.e.mean % increase in R_{aw} was determined from 3 control histamine challenges. Vanadate was infused at 0.04 mg kg^{-1} min^{-1} for 35 min (ie. 1 mg kg^{-1} after 25 min) and the histamine response at 25 min and 35 min determined (↑), then expressed as the % increase above the basal R_{aw} value before histamine (O). Histamine responses were also determined after the cessation of vanadate infusion and the % increase in R_{aw} above the basal value ascertained (NB: same animal as in Figure 2). (b) Effect of histamine on dynamic lung compliance (C_{dyn}, ml cmH$_2$O^{-1}) before and after vanadate in the same animal. The decrease in C_{dyn} in response to histamine challenge (↓) was expressed as a % decrease below the basal C_{dyn} value (O).

Table 1 Hyperreactivity to histamine after vanadate (dose-response relationship of reactivity ratio)

[Vanadate] (mg kg^{-1})	n	*Reactivity ratio*	
		R_{aw}	C_{dyn}
0		1.0	1.0
0.3	3	1.47 ± 0.17	1.32 ± 0.20
1	5	1.95 ± 0.08	1.79 ± 0.14
3	3	2.26 ± 0.14	1.80 ± 0.29

Values show mean ± s.e.mean reactivity ratios for R_{aw} and C_{dyn} for histamine (3 μg kg^{-1}) after vanadate (0.3–3 mg kg^{-1} infused over 25 min).

vanadate infusion and reversal after cessation of infusion was also observed with these agents.

As well as increasing the reactivity to histamine, as measured by the R_{aw} and C_{dyn} changes, vanadate also augmented the transient tachypnea occurring on histamine challenge (see Figure 2). Vanadate increased the tachypnea to histamine, i.e. tachypnoea ratio >1, in a dose-related manner in association with the increased reactivity (in terms of R_{aw}) of the airways ($r = 0.66$, Figure 4), with only 1 in 11 animals showing a fall in the extent of tachypnea to histamine after vanadate, ie. a tachypnoea ratio <1.

The reactivity to histamine was also measured after exposure to an aerosol of vanadate. Hyperreactivity to histamine developed after either a 1 or 3 min exposure to a 10% (w/v) vanadate aerosol (see Methods). After a 1 min exposure histamine reactivity was unchanged for R_{aw} (0.83 ± 0.17 × control) but increased for C_{dyn} (1.47 ± 0.17 × control, $n = 3$). Reactivity was further enhanced after a 3 min aerosol exposure, to 1.62 ± 0.35 × control for R_{aw} and 1.83 ± 0.21 × control ($P < 0.02$) for C_{dyn} ($n = 6$), and was abolished 15 min after exposure. The osmotically equivalent saline solution given for 3 min as an aerosol produced no consistent changes in histamine reactivity in 3 animals.

Effect of vagotomy, atropine and propranolol on vanadate-induced hyperreactivity

The possible involvement of a vagally-mediated reflex and/or of the cholinergic system in the actions of vanadate (1 mg kg^{-1}) was investigated. In artificially respired animals with bilaterally sectioned cervical vagi (vago-sympathectomy) the response to histamine (3 μg kg^{-1}) was greatly reduced, therefore 9 μg kg^{-1} histamine was used to approximate the degree of bronchoconstriction seen with the vagi intact. R_{aw} was increased by 105 ± 49% and C_{dyn} decreased by 26 ± 6% in these animals ($n = 4$). At the end of vanadate infusion the hyperreactivity to histamine was not different from that in non-vagotomized animals, with a reactivity ratio for R_{aw} of 1.80 ± 0.14 × and for C_{dyn} of 2.19 ± 0.51 × control in these 4 animals, although the effect on C_{dyn} tended to be slightly greater than with the vagi intact.

Atropine (1 mg kg^{-1}), in non-vagotomized spontaneously breathing animals, reduced the response to histamine and so histamine challenge was increased from 3 to 6 μg kg^{-1} (see Methods). Atropine had no effect on the enhancement of reactivity to increased histamine by vanadate for R_{aw} (reactivity ratio = 1.86 ± 0.27), but did partially diminish the reactivity increase for C_{dyn} (reactivity ratio = 1.31 ± 0.03, $n = 3$, $P < 0.02$). Propranolol increased the control bronchoconstrictor response to 3 μg kg^{-1} histamine before vanadate treatment but had no effect

Table 2 Effect of vanadate on reactivity to histamine, acetylcholine and 5-hydroxytryptamine

Drug ($\mu g\,kg^{-1}$)	R_{aw}		C_{dyn}	
	Control % increase	*Reactivity ratio*	*Control % decrease*	*Reactivity ratio*
Hist (3)	79 ± 30%	1.95 ± 0.08	30 ± 8%	1.79 ± 0.14
5-HT (6)	63 ± 11%	3.27 ± 0.57	36 ± 7%	1.64 ± 0.22
ACh (6)	94 ± 13%	1.57 ± 0.03	31 ± 10%	1.41 ± 0.21
Sensitivity rank:	5-HT > Hist > ACh		Hist > 5-HT > ACh	

Values show mean ± s.e.mean control changes in R_{aw} and C_{dyn} (% increase or decrease, respectively) to histamine (Hist, $3\,\mu g\,kg^{-1}$, $n = 5$), 5-hydroxytryptamine (5-HT, $6\,\mu g\,kg^{-1}$, $n = 4$) and acetylcholine (ACh, $6\,\mu g\,kg^{-1}$, $n = 3$) and reactivity ratio to each agent after $1\,mg\,kg^{-1}$ vanadate.

on the subsequent enhanced reactivity to histamine after vanadate for R_{aw}, but the reactivity ratio for C_{dyn} was decreased to $1.27 \pm 0.11 \times$ control ($P < 0.05$, $n = 3$). The control decrease in C_{dyn} in response to histamine before vanadate, but after propranolol treatment, was $62 \pm 5\%$ ($n = 3$). This was much greater than the normal decrease of $30 \pm 8\%$ ($n = 5$) in untreated animals and was outside the range for consistent enhancement of reactivity by vanadate

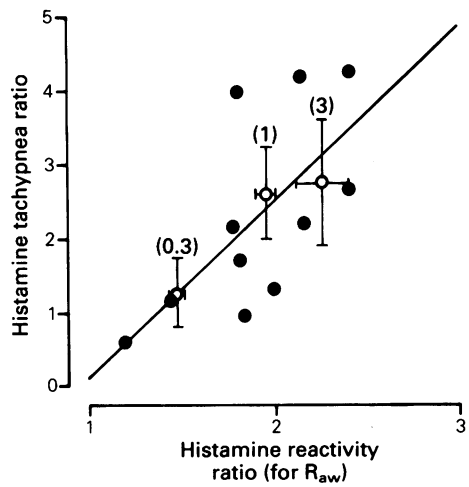

Figure 4 Relationship between histamine tachypnea ratio and histamine reactivity ratio (in terms of R_{aw}; $3\,\mu g\,kg^{-1}$ histamine) with increasing doses of vanadate ($0.3-3\,mg\,kg^{-1}$ infused over 25 min). Data from individual animals (●) shows significant correlation between tachypnoea and reactivity ratios, $r = 0.66$, $P < 0.05$ for 11 animals. Points showing mean ± s.e.mean of tachypnea ratio and reactivity ratio (for R_{aw}) to $3\,\mu g\,kg^{-1}$ histamine for each vanadate dose are also shown (○) with the vanadate dose (in $mg\,kg^{-1}$) indicated above each point, with 3 or more animals in each group (see Table 1).

to the usual $1.79 \times$ control response found in the untreated control animals. We suggest that this accounts for the apparent fall in the C_{dyn} reactivity ratio after propranolol.

Discussion

Vanadate produced a bronchoconstriction and airways hyperreactivity (to several agents) after intravenous infusion. Only a small degree of bronchoconstriction could be demonstrated, however, after a short exposure to an aerosol of vanadate, but at the same time an increased bronchoconstrictor reactivity to histamine could readily be shown. Both these effects were rapid in onset and dose-dependent and were also rapidly reversed following cessation of exposure.

The bronchoconstriction appeared to relate to a direct effect of vanadate on airways smooth muscle because bilateral vagotomy, atropine or propranolol pretreatment did not antagonize its action. This is compatible with *in vitro* evidence (Nayler & Sparrow, 1983) that contraction involves a direct action on airways smooth muscle independent of secondary mediators, an effect that may occur through mobilization of intracellular Ca^{2+}, perhaps by inhibiting membrane Ca-ATPase activity.

The airways hyperreactivity produced after vanadate exposure was relatively non-specific in that responses to all three bronchoconstrictors tested were enhanced. There was, however, a difference in the degree of enhancement of bronchoconstriction to each agent following vanadate, with a sensitivity ranking of 5-HT > histamine > ACh. As with the bronchoconstriction the hyperreactivity was rapid in onset and the extent was dose-related; it was also quickly reversed after cessation of exposure. The increased reactivity could not be explained by differences in functional residual capacity (FRC) after vanadate that could produce changes in R_{aw} and C_{dyn} values and an ostensible change in reactivity. In fact, the FRC

increase to histamine after vanadate was never less than that before vanadate (see Figure 2) and this would lead to an underestimation of the extent of the change in reactivity.

The mechanisms responsible for this hyperreactivity, at least in terms of the response to histamine, appeared to be non-vagal (efferent) in origin. At low histamine concentrations ($3\,\mu g\,kg^{-1}$) the bronchoconstriction in our experiments was largely vagally-mediated via the irritant receptor reflex (Mills & Widdicombe, 1970) and was greatly diminished after bilateral vagotomy. Increasing histamine concentration to $9\,\mu g\,kg^{-1}$ restored the bronchoconstrictor action and the changes in R_{aw} and C_{dyn} were still enhanced in these animals by vanadate. In this situation the bronchoconstriction to histamine appears to be due to a direct action of histamine on airways smooth muscle and possibly other action(s) within the lung (e.g. on capsaicin-sensitive neurones, i.e. afferent C-fibre nerves; Martling *et al.*, 1984). The observation that vanadate-induced hyperreactivity does not rely on the integrity of the vagus indicates that the site through which the hyperreactivity is initiated is in the periphery, i.e. in the lung itself. The hyperreactivity could not be attributed to an action of vanadate to increase cholinergic transmitter release since the hyperreactivity was still present in atropine-treated animals, although the change in C_{dyn} was now diminished. This implies a minor involvement of a cholinergic component in the hyperreactivity seen in the lung periphery, where responses are reflected largely by the C_{dyn} measurements. Thus, histamine responses arising predominantly via cholinergic (vagal) pathways, i.e. at low histamine concentrations, and via non-cholinergic pathways, i.e. at higher histamine concentrations, were similarly enhanced.

An effect of vanadate on the sympathetic component of the reflex response to histamine was examined since vanadate can chelate noradrenaline (Cantley *et al.*, 1978a). Such an action could modify the sympathetic inhibitory component of the histamine reflex response and lead to the observed hyperreactivity. This was ruled out using propranolol where complexing of noradrenaline would now no longer change the response to histamine. Thus, a major role for either division of the autonomic nervous system could not be demonstrated in the hyperreactivity seen after vanadate exposure.

Possible sites through which vanadate might act within the lung to enhance reactivity include the airways smooth muscle itself or via an effect on afferent C-fibre neurones (e.g. on an axon reflex pathway), although an action at other sites or via secondary mediators cannot be excluded. The production of hyperreactivity after both aerosol and i.v. vanadate is compatible with an action at either or both these sites. The mechanism (or the relative components) of airways hyperreactivity to 5-HT and ACh may vary from that to histamine since the mode of action of each bronchoconstrictor is different (as with differing concentrations of histamine).

Aside from the enhancement of airways reactivity, vanadate also increased the tachypnoea to histamine (see Figure 4) and the other bronchoconstrictors (data not shown). For histamine the tachypnoea, associated largely with irritant receptor and possibly C-fibre stimulation (Coleridge *et al.*, 1978; 1981), was related to vanadate concentration, and hence also to hyperreactivity, and possibly reflected an increase by vanadate in afferent activity of the irritant receptors or afferent C-fibres stimulated by histamine. The increase in tachypnoea may also have reflected a central action of vanadate, although centrally mediated effects on blood pressure and heart rate in dogs occur only after intracerebroventricular vanadate administration and not after i.v. challenge (Hom *et al.*, 1982). The possible effects on afferent nerves, or on central control, may occur via an action of vanadate in inhibiting membrane Na^+/K^+-ATPase activity (Cantley *et al.*, 1977; 1978b), which could alter nervous activity. A similar change in afferent activity could also explain the production of airways hyperreactivity. While the two phenomena of increased reactivity and tachypnoea were associated, the hyperreactivity could not be attributed to the increased tachypnoea, since it was unaltered in mechanically respired animals (i.e. in vagotomy experiments) with a constant rate of ventilation.

In summary, the evidence presented suggests that vanadate-induced bronchoconstriction is related to an action of vanadate on airways smooth muscle itself. The hyperreactivity appears to occur independently of activity in efferent vagosympathetic pathways and it is suggested that the site of action of vanadate in inducing hyperreactivity is also within the lung (e.g. on airways smooth muscle, via afferent C-fibre nerve pathways, i.e. an axon reflex, or via secondary mediators).

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