J. Pharm. Pharmacol. 1988, 40: 443-445
Communicated August 18, 1987

Enhancement of capsaicin-induced contraction of guinea-pig tracheal smooth muscle by vanadate

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Abstract—Contractions of guinea-pig isolated tracheal smooth muscle by submaximal capsaicin (0·1 μM) were enhanced by 43% ($P < 0.01$) after vanadate (10 μM for 10 min) preincubation. Contractile responses to acetylcholine (0·1–100 μM), histamine (1–100 μM) or substance P (0·01–1 μM) were, in contrast, not affected by prior vanadate exposure. It is suggested that tachykinin release from capsaicin-sensitive afferent nerve endings within the airways was enhanced by vanadate while airway smooth muscle reactivity remained unchanged.

Vanadate, the commonly occurring pentavalent form of the element vanadium, produces bronchoconstriction and airways hyperreactivity in man after exposure to airborne vanadate compounds. This can occur after a single exposure in non-atopic individuals, suggesting a direct action on the airways (U.S. Department of Health, Education & Welfare Report 1977; Musk & Tees 1982). In guinea-pigs, intravenous (i.v.) vanadate has been shown to cause a dose-related bronchoconstriction which arises via a direct action on airways smooth muscle (Nayler & Mitchell 1987). This is consistent with the in-vitro action of vanadate in contracting isolated intact airways smooth muscle of the guinea-pig from both central and peripheral airways, apparently by mobilizing intracellular Ca^{2+} (Nayler & Sparrow 1983). Tracheal, and other, smooth muscle preparations from monkey, rabbit and rat are also directly contracted by vanadate (Ueda et al 1985; Ishii & Shimo 1985; Hudgins & Bond 1981).

Airways hyperreactivity to several bronchoconstrictors, histamine, acetylcholine (ACh) and 5-hydroxytryptamine, also occurs after exposure to either i.v. or aerosolized vanadate in guinea-pigs. This effect arises independently of vagosympathetic (efferent)/cholinergic pathways and the site of action appears to be within the lung itself (Nayler & Mitchell 1987). Increased smooth muscle reactivity to several spasmogens after vanadate has been demonstrated in rats both in-vitro and in-vivo. Vanadate administered in drinking water enhanced vascular reactivity in-vivo to noradrenaline and angiotensin II, with no sustained increase in blood pressure to vanadate noted (Jadhav & Jandhyala 1983). Contractile responses of rat isolated tracheal smooth muscle to ACh and KCl were augmented by vanadate at concentrations below threshold for producing contraction itself, possibly by inhibition of Ca-ATPase activity to decrease Ca^{2+} translocation (Ishii & Shimo 1985).

Vanadate can also influence smooth muscle tone by neurogenic mechanisms. Spontaneous contractile activity in isolated intestinal smooth muscle is initially inhibited by vanadate and this is followed by enhanced contraction (Hudgins & Bond 1981). The initial inhibitory response appeared to involve the release of an inhibitory transmitter, a catecholamine, from intramural sympathetic nerve endings or enterochromaffin cells.

The aim of the present experiments was to determine whether the increase in airways reactivity seen in-vivo in the guinea-pig reflected an effect of vanadate on airways smooth muscle reactivity to other spasmogens or some other action within the lung. This was investigated using guinea-pig tracheal smooth muscle by comparing reactivity to several spasmogens before and after vanadate pretreatment in-vitro.

Methods

Guinea-pigs (300–450 g) were stunned and exsanguinated. The trachea was excised and placed in a modified Krebs solution at 30–35°C. Its composition was (mM): NaCl 119·8, KCl 4·7, $NaHCO_3$ 14·9, $CaCl_2$ 2, dextrose 11, KH_2PO_4 1·18 and K_2H_2EGTA 0·05. The solution was buffered to pH 7·3 with NaMOPS 5 mM (titrated with NaOH to pH 7·3). Single rings were dissected from the trachea and opened opposite the trachealis muscle. Threads were attached to each end and the preparation was mounted in a 20 mL organ bath. Isometric tension was measured using a Grass FTO3 force transducer connected to a Rikadenki chart recorder and a load of 0·4–0·6 g initially applied. Preparations were equilibrated for at least 1 h with the bathing solution being changed at 20–25 min intervals. The Krebs solution was continuously gassed with 95% O_2/5% CO_2 and maintained at 37°C.

The effect of vanadate (10 μM preincubated for 10 min) on ACh (0·1–100 μM)-, histamine (1–100 μM)- and substance P (0·01–1 μM)-induced contractions was compared with control responses on the same tissue. Capsaicin responses diminished with each challenge (data not shown), so control responses to capsaicin (0·01–1 μM) and those after vanadate were determined on different tissues. Contractile responses to 100 μM ACh were used as a reference standard for comparison of responses between each preparation. Since only single concentrations of agonist were tested on any one tissue, an estimate of the EC50 value for contraction was obtained by interpolation between the mean data points.

Drugs and chemicals. Vanadate solutions ($NaVO_3$, Hopkins and Williams) were prepared daily and had a pH of 7·2. The following drugs (Sigma) were used; histamine diphosphate, acetylcholine chloride, substance P and capsaicin (8-methyl-*N*-vanillyl-6-nonenamide). Capsaicin was initially dissolved in ethanol and diluted 1:5 in saline (0·9% NaCl) to give a stock concentration of 100 mg mL^{-1}. Further dilutions were made in water to give a working stock of ≤ 0.1 mg mL^{-1} capsaicin. All chemicals used were analytical grade and solutions were made with double deionized water.

Statistics. Results are expressed as the mean ± s.e.m. of 3 or more preparations (n) obtained from at least 2 animals. Statistical evaluation was performed by analysis of variance with $P < 0.05$ regarded as significant.

Results

Vanadate (10 μM) preincubated for 10 min produced little or no change in basal isometric tension exerted by the tracheal rings (no more than 10% of the tension to a reference 100 μM ACh contraction). Contractions to single concentrations of ACh (0·1–100 μM), histamine (1–100 μM) or substance P (0·01–1 μM) were not altered after this vanadate pretreatment (Fig. 1a). In contrast, the contractile response to 0·1 μM capsaicin (~EC50

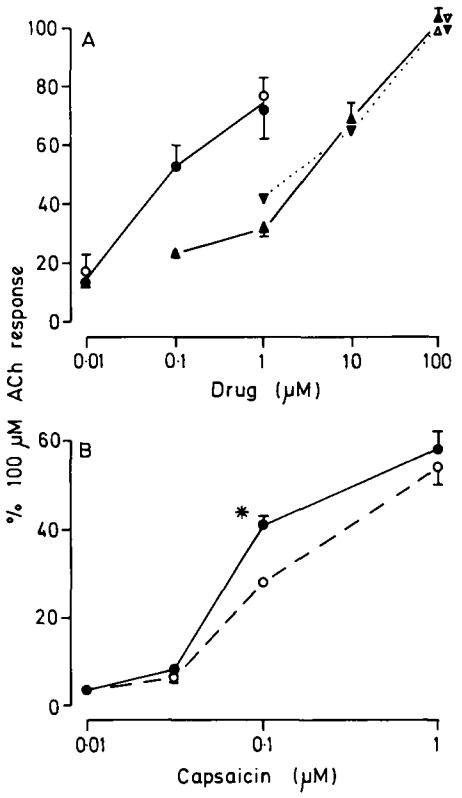

FIG. 1. (A) Contractile response of isolated tracheal smooth muscle to histamine (▽,▼), ACh (△,▲) and substance P (○,●) in the absence (open symbols) and presence (filled symbols) of vanadate (10 μM preincubated for 10 min). Full concentration-response curves were performed for each agonist, although some control values are hidden behind their respective test values (ie. filled symbols). Responses are expressed as a % of the control tension to 100 μM ACh in the same tissue. Points are mean ± s.e.m. of 3 or more tissues. (B) Contractile response of isolated tracheal smooth muscle to capsaicin in the absence (○) and presence (●) of vanadate (10 μM preincubated for 10 min). Responses are expressed as a % of the control tension to 100 μM ACh in the same tissue. Points are mean ± s.e.m. of 3 or more tissues (*$P < 0.01$).

dose of capsaicin) was significantly enhanced by 43% by prior vanadate incubation ($P < 0.01$, $n \geq 6$, Fig. 1b). The estimated EC50 value for capsaicin (see Methods) after 10 μM vanadate was half that of the control group, with a value of 0·05 μM (vs 0·1 μM in the control group). Maximum contractile responses to capsaicin (1 μM) were no different in tissues from the control or vanadate exposed groups. A lower concentration of vanadate (1 μM) did not significantly enhance the response to 0·1 μM capsaicin ($P > 0.05$, data not shown), while concentrations of vanadate > 10 μM were not tested because of the contraction production (Nayler & Sparrow 1983).

Discussion

In the guinea-pig isolated trachea, vanadate, at a threshold concentration for producing contraction itself, induced potentiation of the contractile response to a submaximal concentration of capsaicin. This paralleled the in-vivo situation in the guinea-pig where doses of vanadate producing only a small bronchoconstriction caused a significant increase in airways reactivity to other bronchoconstrictors (Nayler & Mitchell 1987). Contractile responses in-vitro to histamine, ACh and substance P over the complete dose-response range, in contrast, remained unchanged by prior vanadate treatment. Contraction of isolated airways smooth muscle by these three agents occurs via direct activation of specific receptors on the smooth muscle cell membrane; the lack of effect of vanadate on these responses suggests that the inherent responsiveness of the tracheal smooth muscle was not altered by this pretreatment regime. This differs from the response seen in rat trachea where vanadate increased smooth muscle reactivity to ACh and KCl (Ishii & Shimo 1985).

Capsaicin-induced contraction of airways smooth muscle is brought about by activation of chemosensitive C-fibre nerves located within the wall of the tissue and the release of tachykinins, including substance P, from these nerve endings (Saria et al 1983; Lundberg et al 1984). Capsaicin pretreatment in guinea-pigs induces a loss of substance P immunoreactivity from afferent C-fibres within the lung (Lundberg & Saria 1982; Lundberg et al 1983a; Martling et al 1984) and the contractile response of the trachea and bronchus to capsaicin is lost in these animals. Substance P, histamine or ACh induced contractions, however, remain unaltered (Lundberg & Saria 1982; Martling et al 1984). Capsaicin-induced bronchial smooth muscle contraction is also inhibited by putative substance P (tachykinin) antagonists (Lundberg et al 1983b). In-vitro activation of afferent C-fibres and release of tachykinins in the guinea-pig trachea by capsaicin does not appear to involve antidromic action potential conduction since tetrodotoxin (TTX) does not block contraction (Lundberg et al 1984). Substance P release in-vitro from spinal cord slices and guinea-pig ureter by capsaicin is also TTX-resistant (Gamse et al 1979; Saria et al 1983).

It seems unlikely that vanadate might increase the efficacy of other tachykinins released along with substance P from these nerve endings onto tracheal smooth muscle, since responses to maximal capsaicin remained unaltered. Vanadate, at a threshold concentration for direct contractile activity, thus appears to potentiate tachykinin release from capsaicin-sensitive nerves within the trachea, after stimulation by capsaicin in-vitro. Capsaicin-induced release of substance P from spinal cord slices is dependent on extracellular Ca^{2+} (Gamse et al 1979) and since vanadate is a potent inhibitor of both Ca-ATPase and Na, K-ATPase activity in several cell types (Cantley et al 1977, 1978; Caroni & Carafoli 1981) including squid axons in-vitro (DiPolo et al 1979), it may act by elevating or maintaining Ca^{2+} levels within these nerve terminals after activation by capsaicin, or by altering the excitability of the nerve cell membrane. Whether vanadate enhances the activity of capsaicin-sensitive (afferent C-fibre) nerves of the airways in-vivo, where antidromic action potential conduction appears to play an important role in tachykinin release (Lundberg & Saria 1983), remains to be assessed. Thus, airways hyperreactivity in the guinea-pig in-vivo after vanadate does not appear to arise via an alteration of airways smooth muscle reactivity, however, it may reflect an action on reflex nervous activity (eg. via capsaicin-sensitive nerves) within the lung.

This work was supported by The Asthma Foundation of Western Australia and a University of Western Australia Special Research Grant. The author would like to thank Drs H. W. Mitchell and M. P. Sparrow for their helpful comments.

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J. Pharm. Pharmacol. 1988, 40: 445–447
Communicated September 26, 1987

Propranolol uptake with high capacity by rat perfused lung

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Abstract—Lung isolated from 7-week-old rats was perfused with pH 7·4 Krebs-Ringer bicarbonate buffer solution (35 mL) containing 1 to 100 $\mu g\ mL^{-1}$ of propranolol and 3% BSA at the recirculation rate of 8 $mL\ min^{-1}$. Almost parallel bi-exponential drug concentration-time curves were obtained at the initial load lower than 10 $\mu g\ mL^{-1}$, whereas relatively slow, mono-exponential decline was found after perfusion at 100 $\mu g\ mL^{-1}$. Pharmacokinetic analysis for the perfusate propranolol concentration-time curves when loaded at 1 to 10 $\mu g\ mL^{-1}$ yielded almost comparable values for the pulmonary perfusion clearance ($0{\cdot}387 \pm 0{\cdot}092$ to $0{\cdot}486 \pm 0{\cdot}095$ $mL\ min^{-1}\ g^{-1}$). In contrast, this parameter was significantly reduced at 100 $\mu g\ mL^{-1}$ ($0{\cdot}113 \pm 0{\cdot}042$ $mL\ min^{-1}\ g^{-1}$). The present findings suggest a trend towards saturation kinetics in the in-vitro pulmonary clearance of propranolol.

In our previous reports, it has been suggested that the first-pass pulmonary elimination of propranolol after the intravenous administration to rats may be driven predominantly by the rapid and extensive uptake by the lung (Iwamoto et al 1987) and may have a particular age-dependence (Iwamoto et al 1988a). However, the detailed mechanism and kinetics of pulmonary propranolol clearance, including the magnitude of its capacity (i.e. saturation kinetics) to extract the drug from the circulation, were not clarified yet in any animal species. This was simply because high enough dose to yield a saturation of its pulmonary clearance was not accessible in-vivo (Pang et al 1982; Roth 1984; Iwamoto et al 1987).

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Well-designed in-vitro organ perfusion experiments may enable us to predict some possible mechanisms or kinetics for the dose- or age-dependent organ clearance of drugs by modifying physiological or pharmacological condition such as flow rate or initial drug level in the perfusate. Our previous work has proposed the prerequisite of the in-vitro perfusion condition for examining propranolol uptake by the rat lung, i.e. to recirculate the isolated organ with pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3% bovine serum albumin at the flow rate of 8 $mL\ min^{-1}$ (Iwamoto et al 1988b).

The present work was, therefore, designed to estimate an approximate magnitude of the in-vitro pulmonary capacity of 7-week-old rats to clear propranolol from the perfusate by analysing the perfusate drug concentration-time curves after the various initial loads.

Materials and methods

Materials. Propranolol hydrochloride (racemate) was donated by I.C.I-Pharma, Ltd. (Osaka, Japan). Bovine serum albumin (BSA, fraction V) was purchased from Sigma Chemical Co. (St. Louis, USA). Spectrapor membrane tubing (Type 2, MW 12000–14000), used for equilibrium dialysis of propranolol bound to BSA, was purchased from Spectrum Med. Ind. Inc. (Los Angeles, USA). All other chemicals including n-heptane and iso-amylalcohol used to extract unchanged propranolol from its metabolites were of analytical grade.