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Actions of vanadate on vascular tension and sodium pump activity in cat isolated cerebral and femoral arteries

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1 The mechanisms involved in the responses induced by sodium vanadate ($Va_3\ VO_4$) on cat cerebral and femoral arteries were studied. The possibility that these responses were due to Na^+, K^+-ATPase inhibition was investigated by measuring the effect of vanadate on [^{3}H]-ouabain binding to arterial membrane fractions, K^+-induced vasodilatation and ouabain-sensitive $^{86}Rb^+$ uptake.

2 The vanadium compounds (Na_3VO_4, $VOSO_4$, VCl_3 and O_5V_3) induced similar, concentration-dependent contractions in each kind of artery, the cerebral vessels being the most sensitive to these compounds.

3 Exposure of the arteries to a low-Na^+ (25 mM) solution suppressed the contraction caused by vanadate in femoral but not in cerebral arteries.

4 Vanadate-induced contractions were reduced in Ca^{2+}-free medium but remained unaffected by 3×10^{-6} M phentolamine, reserpine pretreatment or 3×10^{-6} M verapamil in both kinds of artery.

5 The addition of 7.5 mM K^+ to the arteries immersed in a K^+-free solution induced vasodilatation, which was not modified by 10^{-3} M vanadate.

6 The consecutive administration of ouabain (10^{-4} M) and vanadate (10^{-3} M) (or *vice versa*), or the simultaneous administration of both agents (10^{-8} to 10^{-3} M) appeared to produce an additive contraction in both types of artery.

7 Vanadate (10^{-7} to 10^{-3} M) did not displace the [^{3}H]-ouabain binding to arterial membrane fractions of these arteries, whereas 10^{-4} M ouabain did.

8 In both kinds of artery, total $^{86}Rb^+$ uptake was reduced by ouabain (10^{-8} to 10^{-3} M), in a concentration-dependent manner, whereas it was not modified by vanadate (10^{-8}–10^{-3} M).

9 These results suggest that vanadate induces contraction in both types of artery by a mechanism unrelated to Na^+, K^+-ATPase inhibition. Such a mechanism is likely to be related to inhibition of the Ca^{2+}-ATPase of the cell membrane and/or the sarcoplasmic reticulum.

Introduction

Vanadium is an element present in small concentrations in serum and tissues of different animals (Nechay, 1984). Under physiological conditions it is present in the + 5 oxidation state, i.e. as vanadate (VO_3^-, VO_4^{3-}) (Nechay, 1984; Erdmann *et al.*, 1984). This anion is able to regulate several biological functions (Nechay, 1984; Erdman *et al.*, 1984). Also, vanadate appears to inhibit Na^+, K^+-ATPase of different tissues, including vascular smooth muscle (Cantley *et al.*, 1977; Beaugé & Glyn, 1978; Bond & Hugdins, 1979; Searle *et al.*, 1983; Nechay, 1984; Erdmann *et al.*, 1984). The cardiac glycosides and vanadate seem to block this enzyme by binding to its extra-and intra-cellular sites, respectively (Cantley *et al.*, 1978). Furthermore, vanadate also has the ability to inhibit Ca^{2+}-ATPase from plasmalemma and sarcoplasmic reticulum of heart and smooth muscle (Wibo *et al.*, 1981; Popescu & Ignat, 1983; Nechay, 1984; Erdmann *et al.*, 1984). Given the important role that these enzymes – particularly the Na^+, K^+-ATPase – play in the regulation of the vascular tone (van

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Breemen et al., 1979; Fleming, 1980; Allen & Navran, 1984), it could be inferred that vanadium compounds play a physiological role in regulating the intracellular ionic concentration and, thereby, the vascular tone. In fact, vanadate induces contractions of vascular (Huot et al., 1979; Ozaki & Urakawa, 1980; Hudgins & Bond, 1981) and non vascular smooth muscles (García et al., 1982; Nayler & Sparrow, 1983; Shimada et al., 1986). However, little is known about the actions of vanadate on cerebral vessels which seem to be endowed with a very active Na^+ pump (Toda, 1980; Marín et al., 1987). Therefore, the aim of the present paper was to analyse and compare the effects of vanadate on cat cerebral and femoral arteries, and to investigate in detail whether these effects were due to a direct action on vascular smooth muscle cells or to noradrenaline release from adrenergic nerve endings. We also considered whether vanadate produces its actions through inhibition of the Na^+, K^+-ATPase, by measuring its effects on [^{3}H]-ouabain binding to membrane fractions of these arteries, K^+-induced relaxation and $^{86}Rb^+$ uptake. These procedures are normally used to characterize the Na^+ pump activity in different vessels (Allen & Navran, 1984). Some of the observed effects were compared to those induced by ouabain, a specific inhibitor of the Na^+ pump.

Methods

Vascular reactivity studies

Cats of either sex (1.5–4 kg) were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) and killed by bleeding. The brain and femoral arteries were removed and the middle cerebral arteries were isolated from the brain. These and the femoral arteries were dissected into cylindrical segments 4 mm in length. Each arterial cylinder was set up for isometric tension recording in an organ bath according to the method of Nielsen & Owman (1971), which has been described in a previous paper (Marín et al., 1987).

A resting tension of 0.5 g and 1 g (optimal resting tone) was applied to cylindrical segments of middle cerebral and femoral arteries, respectively. This tension was readjusted every 15 min during a 90–120 min equilibration period before ionic changes in the medium or addition of drugs were made. The concentration-response curves to vanadium compounds or ouabain were determined in a cumulative manner, at 5 min intervals. When phentolamine or verapamil was administered, with the purpose of inhibiting the contraction caused by vanadate, they were added to the bath 10 min before concentration-response curves to vanadate were made. In order to analyse the influence of extracellular Ca^{2+} on the contractile responses elicited by vanadate, the arterial segments were exposed for 30 min to a Ca^{2+}-free Krebs-Henseleit solution before the administration of vanadate.

[^{3}H]-ouabain binding studies

Once isolated, the cerebral (from the circle of Willis with their branches) and femoral arteries, were immersed in saline solution (0.9% NaCl) at 4°C. In this solution, the connective tissue and blood traces were removed from the vessels, and these were then frozen at − 70°C. When about 1 g of each kind of vessel had been obtained (from several control animals used for different purposes) the binding studies were done according to the method of Gerthoffer & Allen (1981) with minor modifications (Marín et al., 1987).

In order to study the displacement of [^{3}H]-ouabain binding by sodium vanadate (10^{-7} to 10^{-3} M), this was added to the test tubes 10 min before the administration of ouabain (20 nM). Specific binding was defined as binding displaced by 10^{-4} M cold ouabain, which was 80–90% of the total binding.

$^{86}Rb^+$ uptake studies

Cat cerebral and femoral arteries were placed in a Petri dish containing ice-cold Krebs-Henseleit solution (KHS), and in this medium, blood traces and connective tissue were removed. Subsequently, $^{86}Rb^+$ uptake was measured according to the method of Bukoski et al. (1983), which has been described previously (Marín et al., 1987). The arteries were put in vials containing 2 ml of K^+-free, Rb^+-KHS plus ^{86}RbCl (10^{-6} M; specific activity: 4.68 mCi mg^{-1}) for 10 min (time interval in which the uptake was maximum, Marín et al., 1987). Afterwards, the tissues were washed by successive immersion in vials containing 2 ml of K^+-free, Rb^+-KHS for three periods of 30 s. The arteries were then blotted, weighed and digested in vials with 1 ml of H_2O_2 (30% w/v) at 100°C for 5 h. Finally, 2 ml of Ready-Solv HP (Beckman) was added, and radioactivity was measured by use of a liquid scintillation counter (Beckman LS-2800). $^{86}Rb^+$ uptake was expressed as mmol kg^{-1} wet weight. The effect of ouabain (10^{-8} to 10^{-3} M), sodium vanadate (10^{-8} to 10^{-3} M), and ouabain (10^{-8} to 10^{-3} M) plus vanadate (10^{-3} M) on the total $^{86}Rb^+$ uptake was determined. For this purpose these drugs were added to the bath 10 min before and during the incubation period with $^{86}Rb^+$.

Solutions, drugs and statistical evaluations

The composition of the KHS was (mM): NaCl 119, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$. $7H_2O$ 1.2, $NaHCO_3$ 25, glucose 11.1 and the disodium salt of ethylenediamine tetraacetic acid (Na_2EDTA), 0.03. Ca^{2+}-free-KHS was made by omitting $CaCl_2$ in KHS and adding 1 mM ethyleneglycol-bis (β-aminomethyl

eter) N, N'-tetraacetic acid (EGTA). K^+-free-KHS was prepared by omitting KCl and KH_2PO_4 in KHS, but replacing the latter with an equal amount of $NaH_2PO_4.H_2O$ in KHS. K^+-free, Rb^+-KHS was obtained by adding 4.6 mM RbCl in K^+-free-KHS. Low-Na^+ (25 mM) solution was made by replacing NaCl with isosmolar sucrose in KHS.

Ouabain and several vanadium compounds, sodium vanadate (Na_3VO_4; V + 5), vanadyl sulphate ($VOSO_4$; V + 4), vanadium chloride (VCl_3; V + 3) and vanadium pentoxide (V_2O_5; V + 5) were used in this study. Ouabain and vanadium compounds were dissolved in distilled water on the day of the experiment. Stock solutions of noradrenaline, phentolamine and verapamil (10^{-2} M) were made up in saline (0.9% NaCl)-ascorbic acid (0.01%) solution, distilled water and 99.5% ethanol, respectively, and kept at − 20°C. Aliquots of these solutions were diluted just before use with KHS to obtain the desired concentrations. The ouabain and verapamil solutions were protected from light. Reserpine was administered to the animals intraperitoneally 2 and 1 mg kg^{-1} 48 and 24 h, respectively, before the experiments. Control and experimental responses were obtained from separate vascular preparations.

The drugs used were: noradrenaline bitartrate, ouabain octahydrate, sodium vanadate, vanadyl sulphate, vanadium chloride and vanadium pentoxide (Sigma); phentolamine methanesulphonate (Ciba-Geigy); verapamil hydrochloride (Knoll); reserpine (NBC); [^{3}H]-ouabain, and ^{86}rubidium chloride (New England Nuclear). Results are expressed as means ± s.e.means. Deviations from the mean were statistically analysed by use of Student's *t* test; a probability value of less than 5% was considered significant.

Results

Vascular reactivity

All vanadium compounds tested Na_3VO_4, $VOSO_4$, VCl_3 and O_5V_2 induced concentration-dependent contractions in cat cerebral and femoral arteries (Figure 1). The responses elicited by low concentrations of these compounds were small, but they were markedly increased from 10^{-4} M in cerebral arteries and at 10^{-3} M in femoral arteries. Higher concentrations of vanadium compounds precipitated in the bath. The contractions evoked by the four vanadium compounds were similar with the exception of O_5V_2 in femoral arteries, which showed the mildest effect (Figure 1). Therefore, the mechanism of action of these agents was only analysed in the case of vanadate, which has been the most studied by other authors (Nechay, 1984; Erdmann *et al.*, 1984). In both kinds of vessel, vanadate-evoked contractions were unaffected by 3×10^{-6} M phentolamine, 3×10^{-6} M verapamil or pretreatment with reserpine, whereas they were reduced in a Ca^{2+}-free medium (Figure 2).

The exposure of the vessels to a K^+-free medium induced a small and transient contraction in both kinds of artery (Figure 3). The subsequent administration of 7.5 mM K^+, to these vessels previously contracted by 10^{-5} M noradrenaline, induced a vasodilatation (Figure 3) of 236 ± 10 and 486 ± 30 mg, in cerebral and femoral arteries, respectively. The presence of 10^{-3} M vanadate did not modify the relaxation evoked by K^+ (Figure 3).

The reduction of extracellular Na^+ to 25 mM produced a large, but not sustained, tension increase

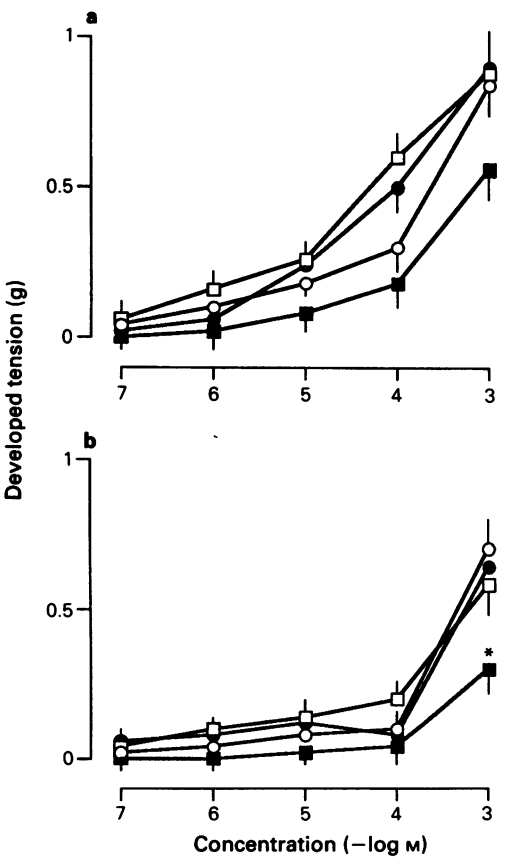

Figure 1 Concentration-dependent curves for sodium vanadate (Na_3VO_4; ○), vanadyl sulphate ($VOSO_4$; ●), vanadium chloride (VCl_3; □) and vanadium pentoxide (O_5V_2; ■) determined in segments of cat middle cerebral (a) and femoral (b) arteries. Each concentration of drug was added at 5 min intervals. Number of arterial segments used for constructing each curve ranges from 4 to 15 ($^*P < 0.05$).

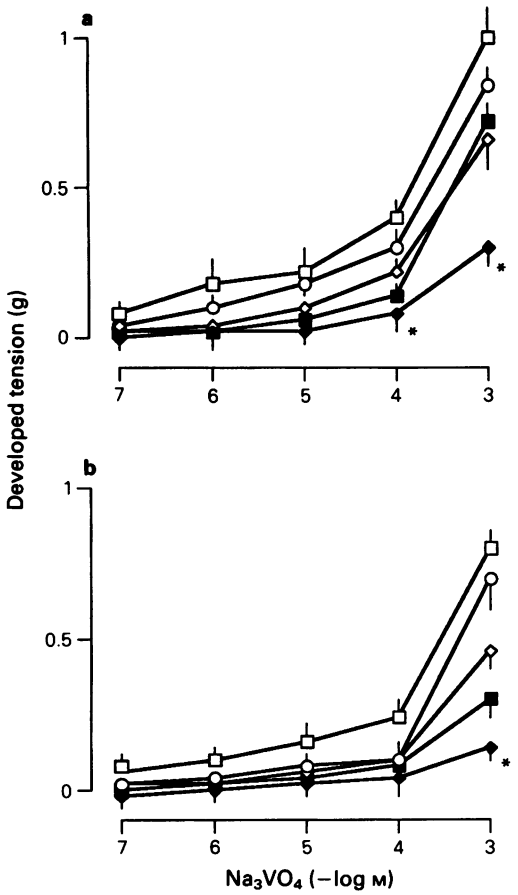

Figure 2 Effect of a 10 min preincubation with either 3×10^{-6} M phentolamine (□) or 3×10^{-6} M verapamil (◇); exposure to a Ca^{2+}-free solution for 30 min (◆), or pretreatment with reserpine (■) on the concentration-response curve for sodium vanadate (Na_3VO_4) in cat middle cerebral (a) and femoral (b) arteries. (○) Control responses to Na_3VO_4. The numbers of arterial segments used to construct the different curves ranged from 4 to 15 (*$P < 0.05$).

the maximal response being 1,310 ± 230 and 2,130 ± 360 mg in cerebral and femoral arteries, respectively. The contraction in brain vessels returned to the basal level within 15 min, but in femoral arteries a residual contraction of 1,210 ± 160 mg remained. In this low Na^+-medium, vanadate (10^{-4} and 10^{-3} M) elicited contractile responses only in cerebral vessels (at 10^{-3} M vanadate induced a response of 430 ± 35 mg, i.e. similar to that obtained in the control situation).

The effects of ouabain plus vanadate were also tested. The administration of 10^{-3} M vanadate on

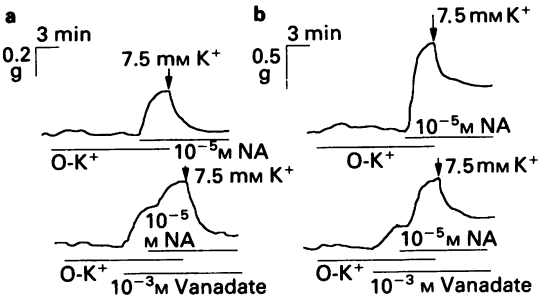

Figure 3 Typical records of the effect of vanadate on K^+-induced relaxation in cat middle cerebral (a) and femoral (b) arteries. Following a period of exposure to a K^+-free solution, noradrenaline (NA) was added to the bath and subsequently 7.5 mM K^+ was added. The time of exposure to different drugs and zero K^+ is indicated by horizontal bars. The experiment lasted 25 min. At least 4 experiments of each type were carried out.

arterial segments previously contracted with 10^{-4} M ouabain, or *vice versa*, induced a further contraction in both types of artery (Figure 4). The simultaneous addition to the bath of the same concentrations of ouabain and vanadate elicited a concentration-dependent contraction in cerebral and femoral arteries (Figure 4), which appeared to be additive at some points on the concentration-response curve.

[^{3}H]-ouabain binding

The characteristics of the specific [^{3}H]-ouabain binding to membranes of cerebral and femoral arteries have been described in a previous paper (Marín *et al.*, 1987). Figure 5 shows the antagonism by cold ouabain (10^{-4} M) of the binding of [^{3}H]-ouabain (20 nM) to these membranes. This binding was not altered by concentrations of vanadate up to 10^{-4} M, while 10^{-3} M vanadate increased it significantly.

$^{86}Rb^+$ uptake

As shown in Figure 6, $^{86}Rb^+$ uptake (10 min incubation) in cerebral and femoral arteries was reduced in a concentration-dependent manner by ouabain (10^{-8} to 10^{-3} M). Vanadate (10^{-8} to 10^{-3} M) did not modify this uptake, nor did it (10^{-3} M) alter the inhibitory effect of ouabain (10^{-8} to 10^{-3} M) in either type of artery.

Discussion

The results obtained in the present study show that different vanadium compounds (Na_3VO_4, $VOSO_4$,

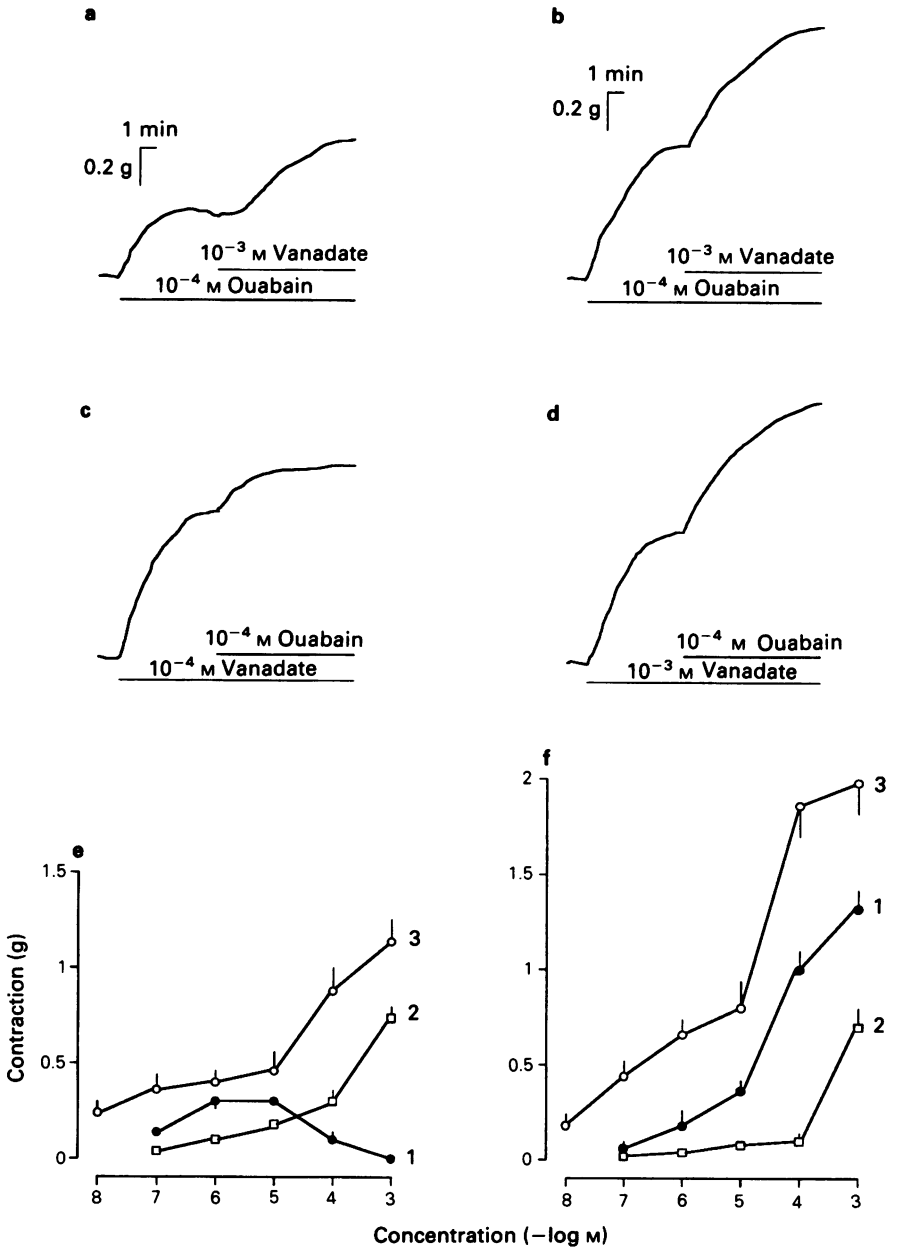

Figure 4 (a–d) Typical records of the effect of vanadate on the contraction induced by ouabain, and *vice versa*, in cat middle cerebral (a and c) and femoral (b and d) arteries. Vanadate was administered 5 min after the addition of ouabain and *vice versa*. (e and f) Concentration-response curves of cerebral (e) and femoral (f) arteries for ouabain (1, ●), vanadate (2, □) and ouabain plus vanadate (3, ○) added simultaneously to the bath. At least 4 experiments of each type were carried out.

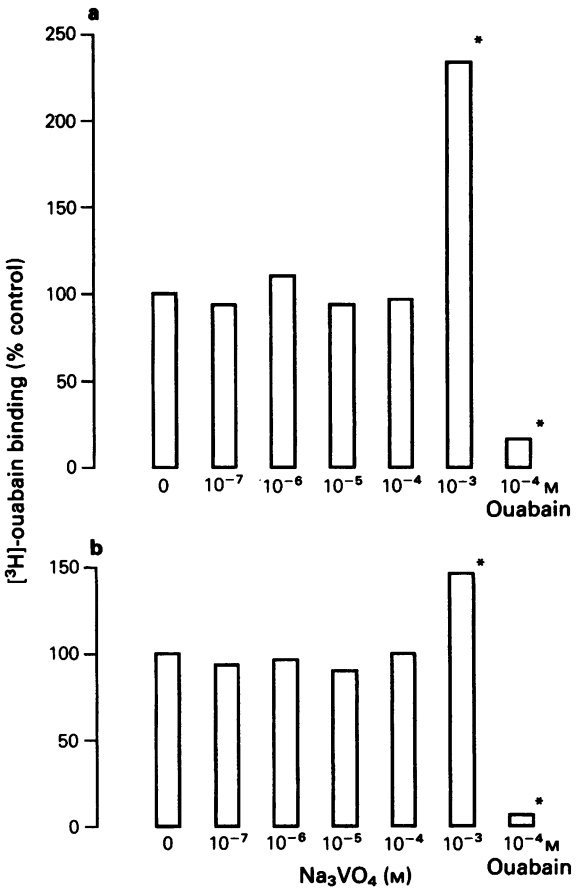

Figure 5 Effect of 10^{-4} M ouabain and different concentrations of vanadate (Na_3VO_4) on [^{3}H]-ouabain binding to crude membranes of cat cerebral (a) and femoral (b) arteries. The vessels were incubated with 20 nM [^{3}H]-ouabain for 60 min. The 100% binding was of 317 and 107 fmol mg^{-1} protein in cerebral and femoral arteries respectively. The experiment was performed in triplicate (*$P < 0.05$).

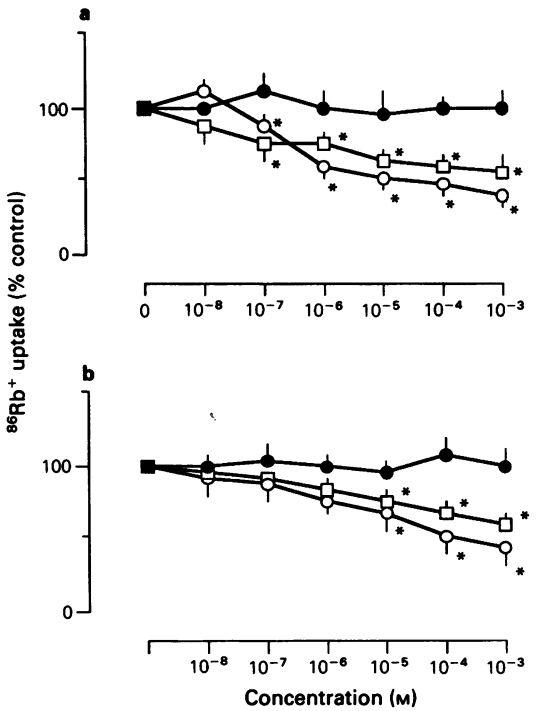

Figure 6 Effect of different concentrations of ouabain (○), sodium vanadate (Na_3VO_4) (●) or ouabain plus 10^{-3} M vanadate (□) on the total $^{86}Rb^+$ uptake in cat cerebral (a) and femoral (b) arteries. The vessels were incubated for 10 min in a medium containing $^{86}Rb^+$ and the drugs were added to the bath 10 min before and during the isotope incubation period. The results are expressed as percentage of the $^{86}Rb^+$ uptake obtained in absence of drugs (100%), which was 2.35 ± 0.46 and 1.55 ± 0.19 mmol $^{86}Rb^+$ kg^{-1} wet weight in cerebral and femoral arteries, respectively. Each point represents the mean of 3–11 paired experiments and vertical lines indicate s.e.mean (*$P < 0.05$).

VCl_3 and O_5V_2) elicited contractile responses which were greater in cerebral than in femoral arteries. The responses obtained with the different vanadium compounds were similar in each type of vessel, with the exception of O_5V_2 in femoral arteries. Therefore, the mechanisms involved in the vascular contractions were studied only in the case of vanadate, which is also the most widely used compound (Huot *et al.*, 1979; Ozaki & Urakawa, 1980; Ueda *et al.*, 1985; Shimada *et al.*, 1986).

The contractions induced by vanadate were unaffected by phentolamine, pretreatment with reserpine or verapamil, even though 10^{-3} M vanadate increases the release of [^{3}H]-noradrenaline from both kinds of artery (Marín *et al.*, 1986). These findings suggest that, although vanadate releases noradrenaline, its contribution to the contraction caused by this agent is small in both types of artery. In other vessels, it was also found that phentolamine (Huot *et al.*, 1979; Ozaki & Urakawa, 1980; Fox *et al.*, 1983; Shimada *et al.*, 1986) or pretreatment with reserpine (Fox *et al.*, 1983) did not significantly alter the contraction induced by vanadate.

Vanadate-induced increases in tension were reduced in Ca^{2+}-free solution, but were not modified by verapamil. This finding indicates that a part of the

response is due to the Ca^{2+} entry into the vascular smooth muscle cells by a mechanism insensitive to Ca^{2+}-antagonists, and the other part depends on the Ca^{2+} release from intracellular stores.

On the other hand, K^+-induced relaxation in both kinds of artery exposed to a K^+-free solution was not changed by vanadate, while it was abolished by 10^{-4} M ouabain (Marín *et al.*, 1987). However, in tracheal smooth muscle, vanadate partially and ouabain completely inhibited this relaxation (Ueda *et al.*, 1985). These findings indicate that the mechanism of action of ouabain and vanadate seems to be different.

The exposure of the vessels to a low-Na^+ medium, in order to reduce the activity of Na^+, K^+-ATPase, caused a small reduction or suppression of the contraction elicited by vanadate in cerebral and femoral arteries, respectively. However, the response induced by ouabain in both types of vessel was abolished (Marín *et al.*, 1987). The lack of response to vanadate of femoral arteries in low Na^+ is probably due to the fact that the low Na^+ medium produced a high remaining contraction, which could interfere with the effect of vanadate. Fox *et al.* (1983) observed that the replacement of Na^+ by Li^+ produced a transient contraction in rabbit aorta and subsequent addition of vanadate induced a contraction almost similar to that found in control solution.

The administration of a high concentration of ouabain (10^{-4} M) or vanadate (10^{-3} M), which would induce an almost complete inhibition of the Na^+-pump, did not abolish the response elicited by subsequent administration of vanadate (10^{-3} M) or ouabain (10^{-4} M), respectively; on the contrary, the response seemed additive (Figure 4). Similar findings were obtained when both drugs were simultaneously administered at the same concentrations. All these results indicate that different mechanisms of action are involved in the response to ouabain and vanadate, the latter being unrelated to the Na^+, K^+-ATPase inhibition. However, Myers & Boerth (1980) found that the administration of ouabain plus vanadate to rabbit left atrial strips displaced to the left the concentration-response curve obtained with either agent alone, but the maximum response was similar. They concluded that both drugs act by the same mechanism, i.e., through Na^+,K^+-ATPase inhibition.

Vanadate neither altered the total $^{86}Rb^+$ uptake nor that produced by different concentrations of ouabain. In vascular smooth muscle cells (Searle *et al.*, 1983) and in dog saphenous vein (Huot *et al.*, 1979), vanadate also failed to reduce $^{86}Rb^+$ uptake. In addition, [^{3}H]-ouabain binding was not modified by vanadate from 10^{-7} to 10^{-4} M; at 10^{-3} M it was increased. In segments of rat caudal and abdominal aorta (Wong *et al.*, 1984) and in intact guinea-pig vas deferens (Wong *et al.*, 1981), vanadate (up to 10^{-4} M) did not alter the [^{3}H]-ouabain binding. These data suggest that this agent and ouabain attach to different sites of Na^+, K^+-ATPase. The increase in [^{3}H]-ouabain binding found in the presence of 10^{-3} M vanadate in both kinds of artery is difficult to explain, but it could be due to an increase in the affinity of binding sites for ouabain and not to an increase in the number of binding sites. This hypothesis was substantiated by Erdmann *et al.* (1979).

In summary, the results of our experiments indicate that the mechanism of action of vanadate in both kinds of artery is similar, and independent of Na^+, K^+-ATPase inhibition. The results are best explained by assuming that vanadate, inhibits the Ca^{2+}-ATPase of the cell membrane and the sarcoplasmic reticulum. Indeed, it has been demonstrated that vanadate blocks this enzyme (O'Neal *et al.*, 1979; Adams & Swartz, 1980; Dupond & Bennett, 1982), which seems to be involved in the Ca^{2+} extrusion (van Breemen *et al.*, 1979). Therefore, its inhibition would lead to an increase in the intracellular levels of the free Ca^{2+}, which would cause the contraction. In other vascular preparations, vanadate-induced contractions also appear to be mediated by inhibition of Ca^{2+}-ATPase (Ozaki & Urakawa, 1980; Shimada *et al.*, 1986). The failure of vanadate to inhibit the Na^+ pump is probably due to the fact that when it enters the vascular smooth muscle cells it binds to intracellular proteins and/or it is converted to the vanadyl ion (Searle *et al.*, 1983), which has a minimal influence on the enzyme (Svoboda *et al.*, 1984).

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