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Vanadate Potentiates Hypoxic Pulmonary Vasoconstriction[1]

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ABSTRACT

Vanadate, an essential trace element and an inhibitor or stimulator of many enzymes, potentiates the hypoxic vasoconstriction in isolated lung preparations. However, the mechanism of action of vanadate in the lung circulation is unclear. We compared, in isolated rat lungs, the effect of vanadate (3×10^{-5} M) on hypoxia-induced vasoconstriction with the vasoconstriction caused by angiotensin II, KCl or NaCN, and found that vanadate preferentially enhanced the hypoxia- and NaCN-induced pressor responses. Vanadate also shifted the stimulus-response curve for oxygen such that vasoconstriction occurred at a higher PO_2 than in control lungs, indicating that vanadate had affected the oxygen sensing mechanism in the lungs. We postulated that vanadate might potentiate hypoxic vasoconstriction, in part, by activating a protein kinase C (PKC), and compared the effect of phorbol myristate acetate (PMA; 5×10^{-8} M) on hypoxic vasoconstriction with that of vanadate. Both agents, PMA and vanadate, potentiated hypoxic vasoconstriction transiently and to a similar degree and the potentiation by both agents was blocked by staurosporine (1 μg/ml), a PKC inhibitor, and 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate, a phospholipase C inhibitor, and partially reduced by the Ca^{++} entry inhibitor nifedipine. We conclude that the similarities between the action of PMA and vanadate in isolated lungs point toward an involvement of the PKC in the mechanism of vanadate-induced potentiation of hypoxic vasoconstriction. In addition, our data indicate that potentiation of hypoxic vasoconstriction by PMA or vanadate may occur, in part, independent of voltage-dependent Ca^{++} entry.

Vanadate and vanadate-containing complexes are of considerable interest as probes of enzymes that catalyze phosphoryl-transfer reactions (phosphorylation) (Ishikawa, 1989; Percival *et al.*, 1990; Ramasarma and Crane, 1981; Xie *et al.*, 1989). Although vanadate, an essential trace element for higher animals, has been shown to be a strong inhibitor (Cantley *et al.*, 1978; DeMaster and Mitchell, 1973; Ishikawa, 1989; Kanaho *et al.*, 1988; Markus *et al.*, 1989; O'Neal *et al.*, 1979) or stimulator (Macara, 1986; Ramasarma *et al.*, 1981; Souness *et al.*, 1985) of many enzymes, the precise mechanism of action of vanadate is currently incompletely understood. Vanadate causes diuresis and natriuresis in the rat (Nechay, 1984; Phillips *et al.*, 1983) and contraction of isolated vascular smooth muscle preparations (Fox, *et al.*, 1983; Shimada *et al.*, 1986) associated with increased calcium influx (Sunano *et al.*, 1988). Some actions of vanadate have been associated with alterations in intracellular calcium pools rather than with facilitated calcium influx, and with changes of the cellular redox state and increased lipid peroxidation (Inouye *et al.*, 1980; Keller *et al.*, 1989; Rosen *et al.*, 1975). In a preliminary report we showed that vanadate potentiates hypoxic pulmonary vasoconstriction in isolated rat lungs (Voelkel *et al.*, 1980) and this has subsequently been confirmed by McMurtry (1984). In this investigation, we examine whether the potentiation of hypoxic vasoconstriction by vanadate is preferential when compared with pulmonary vasoconstriction induced by angiotensin II, potassium chloride or sodium cyanide, a hypoxia mimic, and whether vanadate makes the lung more sensitive to hypoxia (*i.e.*, shifts the dose-response curve for hypoxic vasoconstriction). How vanadate potentiates hypoxic vasoconstriction is unknown. We question whether the potentiation of the hypoxia-induced pressor response by vanadate is due to oxygen free radicals (Keller *et al.*, 1989; Ramasarma *et al.*, 1981) and use oxygen radical scavengers in an attempt to inhibit the effect of vanadate.

Because PMA, an activator of the protein kinase C, has recently been shown to potentiate hypoxic vasoconstriction (Orton *et al.*, 1990; Raffestin and McMurtry, 1987), and because of the pharmacological similarity between the action of PMA and vanadate (Macara, 1986; Montesano *et al.*, 1988), we questioned whether vanadate might act *via* activation of PKC (Brock and Capasso, 1988). If so, then staurosporine, an inhibitor of PKC (Sehic and Malik, 1990; Tamaoki *et al.*, 1986), should prevent the effects of vanadate in the lung. Also, in keeping with a potential role of PKC in vanadate's action in the lung, influx of extracellular calcium may not be as crucial

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ABBREVIATIONS: PMA, phorbolmyristate acetate; PKC, protein kinase C; PLC, phospholipase C; NCDC, 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate; SOD, superoxide dismutase; PSS, physiological salt solution; FIO_2, fraction of inspired oxygen.

in the vanadate-induced potentiation of hypoxic vasoconstriction as in hypoxic vasoconstriction itself (McMurtry *et al.*, 1976). Consistent with a role of PKC, because of the feedback relationship between PKC and PLC activity (Brock and Capasso, 1988; Exton, 1988; Meyer and Stryer, 1988; Nishizuka, 1986; Rhee *et al.*, 1987), we considered that vanadate might also affect PLC activity. We therefore reasoned that the calcium-entry blocker, nifedipine (in a dose with blocked hypoxic vasoconstriction itself), would have little effect on vanadate-induced potentiation of hypoxic vasoconstriction—in contrast to NCDC a PLC inhibitor (Turla and Webb, 1990; N. F. Voelkel *et al.*, in press; Walenga *et al.*, 1980), which we expected to inhibit the vanadate-induced potentiation of hypoxic vasoconstriction.

Methods

Isolated perfused lung studies. Adult male Sprague-Dawley rats with an average weight of 340 ± 40 g were used for all experiments.

After pentobarbital anesthesia (60 mg/kg, i.p.) the lungs were isolated as described previously (McMurtry *et al.*, 1976; Burghuber *et al.*, 1984). The lungs were ventilated with a Harvard Instruments small animal ventilator using a gas mixture of 21% O_2, 5% CO_2 and 74% N_2 and perfused at a constant flow (0.03 ml/g body weight) with heparinized blood that had been collected from three donor rats, or with a physiological salt solution which was composed as follows in mM: 119 NaCl, 4.7 KCl, 5.5 dextrose, 1.17 $MgSO_4$, 15 $NaHCO_3$, 1.18 $KHPO_4$, 50 sucrose and 1.6 $CaCL_2$, 4 vol % Ficoll (MW 70000 Sigma Chemical Co., St. Louis, MO). The purpose of using a physiological salt solution was: a) to investigate the influence of vanadate on the lung without any interference by formed blood elements and b) to eliminate vanadate binding by plasma proteins (Nechay, 1984). After an equilibration period of 30 min at 38°C, each lung was challenged with airway hypoxia and angiotensin II (Sigma Chemical Co., St. Louis, MO) bolus injection into the pulmonary artery line to assure normal vascular reactivity of the preparation. The pulmonary artery pressure was measured with a Statham transducer (Statham Instruments, Oxnard, CA) and recorded continuously on a Gilson recorder (Gilson Medical Electronics, Middleton, WI).

Specific Protocols

Blood perfused lungs. *Effect of vanadate on hypoxic vasoconstriction.* In 11 blood-perfused lungs, responses to various degrees of hypoxia before and after addition of vanadate (3×10^{-5} M) were performed. Because we had found that the potentiating effect of vanadate declines with passage of time (Voelkel *et al.*, 1980), the sequence of the hypoxic challenges was changed in random order from FIO_2 0.21 to either 0.15, 0.10, 0.03 or 0 ($FICO_2$ 0.05, balance N_2). Five different lungs served as time controls; here the sequence of hypoxic stimuli was studied before and after normal saline (instead of vanadate) addition to the reservoir. The time-matched hypoxic responses were plotted against lung effluent PaO_2.

Effect of vanadate on angiotensin II-induced vasoconstriction. In 12 different blood-perfused rat lungs, we performed two angiotensin II and two hypoxic challenges to assure adequate vascular reactivity. Thereafter, in 15-min intervals, bolus injections of increasing doses of angiotensin II (10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} μg/0.1 ml normal saline) were performed before and after addition of vanadate ($n = 8$) and before and after 0.3 ml of normal saline addition ($n = 4$); these latter lungs served as time controls.

Effect of vanadate on potassium chloride-induced vasoconstriction. In eight perfused rat lungs, KCl was added to the blood. First, 2.5 mM was added, and then after a new stable base line was reached, 5 mM. Four of these lungs were pretreated with vanadate (addition of vanadate to the blood reservoir 5 min before first KCl addition), and four lungs were treated with normal saline instead.

Effect of vanadate on cyanide-induced pulmonary vasoconstriction. In four blood-perfused rat lungs, we examined the effect of vanadate addition to the perfusate on the pulmonary vasoconstriction generated by the hypoxia mimic, sodium cyanide. After standard procedures and testing of pulmonary vasoreactivity with AII and hypoxia, NaCN addition to the perfusate to obtain circulating concentration of 10^{-4} M was preceded by vanadate addition to the perfusate. Control lungs ($n = 4$) received normal saline instead of vanadate. The pulmonary vascular pressor response after NaCN and a subsequent hypoxic pressor response (FIO_2 .01) were monitored.

Lungs perfused with physiological salt solution. *Vanadate dose-dependent potentiation of hypoxic vasoconstriction.* A dose-response relationship between vanadate perfusate concentration and magnitude of hypoxic pressor response was established in lungs perfused with physiological salt solution ($n = 10$). This was done as follows: after a 30-min equilibration period, the lung was subjected to two sequences of angiotensin II bolus injection (1 μg/ml) and a 6-min hypoxic stimulation (FIO_2 0.00). Each time interval between angiotensin bolus and onset of hypoxia was 15 min. Then a dose of vanadate was added to the reservoir, the rise in base-line perfusion pressure was recorded and after a stable base line was reached (usually after 3 min), the lung was stimulated with hypoxia (FIO_2 0.00) for 6 min. The sequence of the low and high orthovanadate doses was randomized; altogether we monitored the potentiation of hypoxic vasoconstriction in eight different lungs after 15 separate doses of vanadate. No more than three doses were tested in one lung. The dose of 2.5×10^{-6} M was tested in four lungs, 10^{-5} M was tested in six lungs, 3×10^{-5} M in three lungs and 10^{-4} M in two lungs. Two rat lungs served as time controls, normal saline was added instead of vanadate and the hypoxic stimulation was repeated after saline addition.

Effect of SOD and catalase on vanadate-potentiated hypoxic vasoconstriction. Because of the known catalytic effects of vanadate in reactions that produce active oxygen species (Ramasarma *et al.*, 1981; Rosen *et al.*, 1975), we examined whether the combined administration of SOD (Sigma Chemical Co., St. Louis, MO) (300 U/ml of perfusate) and catalase (Sigma) (7500 U/ml of perfusate) prevented the vanadate-induced potentiation of hypoxic vasoconstriction in lungs perfused with a physiological salt solution. Catalase and SOD were added to the perfusate 3 min before vanadate, and the vasoconstriction after vanadate addition and a subsequent hypoxic challenge (FIO_2 0.00) were monitored ($n = 5$). We had previously shown that these compounds were effective in the isolated rat lung under conditions of oxidant stress (Burghuber *et al.*, 1984; He *et al.*, 1990).

Comparison of the action of PMA and vanadate. In several experiments, we added PMA (10^{-9} – 10^{-8} M) to the lung perfusate and measured the subsequent rise in basal perfusion pressure (*e.g.*, Raffestin and McMurtry, 1987) and then the hypoxic pressure response. In other lungs, we evaluated the dose of the PKC inhibitor, staurosporine, that was required to inhibit the PMA-induced vasoconstriction and the potentiation of the hypoxic pressor response.

We then examined in different lungs whether this staurosporine concentration abolished pulmonary vasoreactivity and found that the angiotensin II pressor response and the hypoxic vasoconstriction were only mildly reduced by this dose of staurosporine (1 μg/ml perfusate or 0.17 μM circulating perfusate concentration). This staurosporine concentration (1 μg/ml) was subsequently used in an attempt to inhibit the vanadate-induced potentiation of hypoxic vasoconstriction (figs. 4 and 5).

Effect of NCDC on vanadate- and PMA-induced responses. Because of the intricate interactions between PLC and PKC (Brock and Capasso, 1988; Nishizuka, 1986), we used NCDC, an agent shown to inhibit PLC (Walenga *et al.*, 1980; Turla and Webb, 1990). NCDC (10^{-5} M) was added to the salt solution perfusate before PMA (5×10^{-8} M) ($n = 4$) or vanadate (3×10^{-5} M) ($n = 5$) and the subsequent change in base-line pressure and magnitude of hypoxic vasoconstriction were monitored.

Effect of nifedipine on PMA and vanadate-induced pulmonary responses. To examine to what extent the hypoxic vasoconstriction and the potentiation of hypoxic vasoconstriction depended on activation of

voltage-dependent Ca^{++} channels and Ca^{++} entry into cells (McMurtry *et al.*, 1976; Sunano *et al.*, 1988), we conducted preliminary studies in seven lungs and found that nifedipine, in a perfusate concentration of 5×10^{-8} M, inhibited hypoxic vasoconstriction by ±70%. This dose of nifedipine also abolished the action of the dihydropyridine analog BAY 2086 (data not shown).

In subsequent experiments, nifedipine was added to the perfusate (to make a circulating perfusate concentration of 5×10^{-8} M) before either PMA (5×10^{-8} M) or vanadate (3×10^{-5} M) addition ($n = 4$ each). Basal pulmonary artery pressure and subsequent hypoxic vasoconstriction (FIO_2 0.00) were measured.

Results

Effects of Vanadate on Baseline Pulmonary Perfusion Pressure and Hypoxic Vasoconstriction in Blood Perfused Lungs

Effect on base-line pulmonary artery pressure. Injection of 0.2 ml of a 10^{-6} M solution of vanadate either as a bolus into the pulmonary artery or into the blood reservoir (circulating concentration 6.6×10^{-5} M) resulted in a transient pressor response (ΔP_{PA} = 2–5 mm Hg, $n = 6$). Addition of vanadate to the reservoir to give a circulating concentration of 3×10^{-5} M before hypoxic stimulation frequently increased the base-line pressure (ΔP_{PA} = 0–5 mm Hg, $n = 15$) when compared with the perfusion pressure before vanadate addition or when comparison was made with time-matched controls where normal saline had been added instead of vanadate.

Effect on oxygen sensitivity. None of the five lungs without vanadate showed any vasoconstriction in response to a FIO_2 of 0.15 (lung effluent PO_2 = 100), whereas all five vanadate-treated, blood-perfused lungs showed hypoxic vasoconstriction when challenged with FIO_2 0.15 (figs. 1 and 2a). The pressure rise at the onset of hypoxic stimulation was more brisk after than before vanadate administration. Vanadate clearly shifted the stimulus-response curve (if one considers the lung effluent PO_2 representative of the alveolar PO_2) (fig. 2a). In contrast, only the pressor responses to doses of angiotensin II greater than 0.1 μg were increased by vanadate (fig. 2b), and the responses to KCl were not affected by vanadate (fig. 2c).

Effect of cyanide-induced vasoconstriction. Cyanide (10^{-4} M) caused a transient pressor response (ΔP_{PA} = 14 ± 2 mm Hg). The shape of the pressor response was reminiscient of the hypoxic pressor response. Pretreatment with vanadate augmented the cyanide-induced vasoconstriction (ΔP_{PA} = 33 ± 4 mm Hg) (fig. 3).

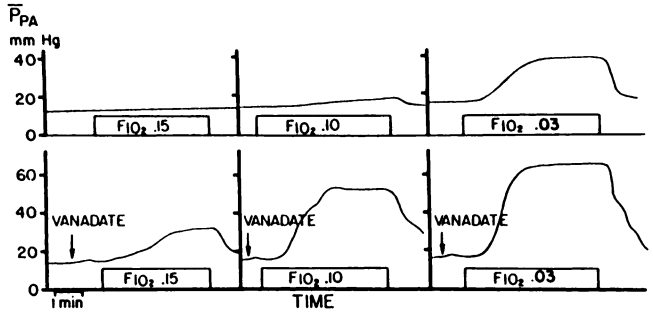

Fig. 1. Effect of Na-orthovanadate on hypoxic pulmonary vasoconstriction. The upper panel shows examples of tracings from a blood-perfused isolated rat lung without Na-orthovanadate; the lower panel shows tracings from a comparable blood perfused lung, however, Na-orthovanadate (perfusate concentration 3×10^{-5} M) had been added immediately before the hypoxic challenge.

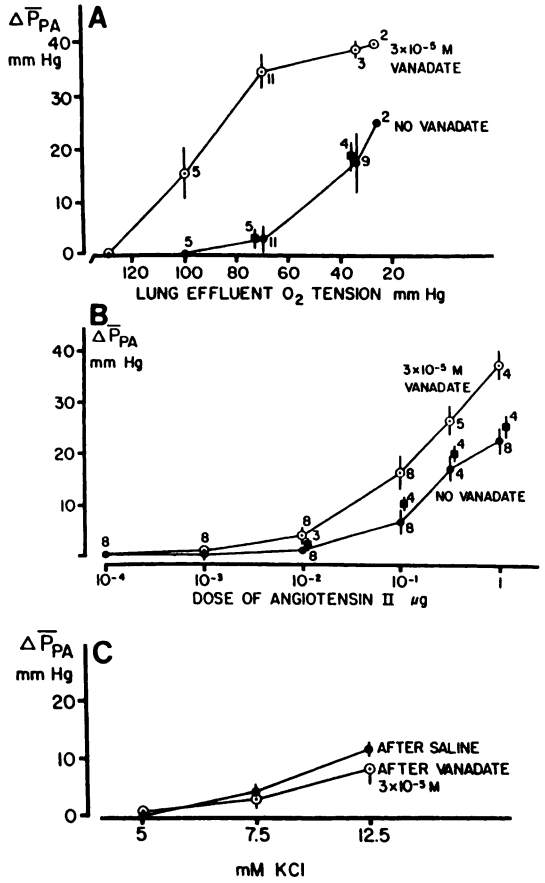

Fig. 2. A) Relationship between the magnitude of the hypoxic pressor response (ΔP_{PA}) and the PO_2 of the pulmonary venous blood. The lungs were ventilated with gas mixtures containing 21, 15, 10, 3 or 0% oxygen (5% CO_2 and balance nitrogen). (●) represent lungs without vanadate addition; (○) lungs with vanadate addition; (■) are lungs without vanadate addition that were time-matched with the lungs that had received vanadate. Numbers represent numbers of rat lungs. B) Effect of vanadate addition to the perfusate on the vasoconstriction due to the bolus of angiotensin II. Symbols as in A. At doses from 10^{-1} to 1 μg of angiotensin II, vanadate significantly augmented the angiotensin response ($P < .01$). C) Effect of vanadate on the pulmonary vasoconstriction in response to KCl addition to the blood perfusate in rat lungs ($n = 4$, KCl after vanadate; $n = 4$, KCl after normal saline).

Potentiation of Hypoxic Vasoconstriction in Lungs Perfused with Cell-Free Physiological Salt Solution (PSS)

Vanadate additions to the reservoir to achieve a circulating concentration greater than 5×10^{-6} M resulted in subsequent hypoxic pressor responses that were potentiated. For a vanadate perfusate concentration of 3×10^{-5} M, the subsequent hypoxic pressor responses were usually 3-fold greater than in controls without vanadate (figs. 4 and 5).

Effect of SOD and catalase on vanadate-induced potentiation of hypoxic vasoconstriction. Addition of SOD plus catalase immediately before vanadate had no effect on the base-line perfusion pressure rise observed after vanadate and did not alter the magnitude of hypoxic vasoconstriction. The hypoxic pressor response was 284 ± 25% of control in vanadate-treated lungs ($n = 5$) and 288 ± 53% of control in vanadate-treated lungs after SOD plus catalase ($n = 5$). Therefore, these

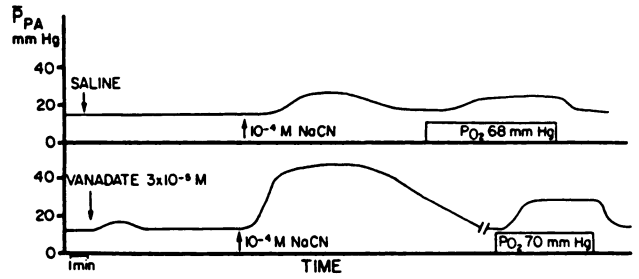

Fig. 3. Effect of vanadate on cyanide-induced pulmonary vasoconstriction in isolated rat lungs perfused with blood. Cyanide caused a transient pulmonary vasoconstriction. The subsequent hypoxic pressor response (FIO_2 0.1) was intact. Addition of vanadate to the perfusate enhanced the cyanide-induced vasoconstriction (PO_2 = effluent oxygen tension).

oxygen radical scavengers did not affect the pulmonary vascular responses after vanadate addition to the PSS perfusate.

Comparison of the effects of vanadate and PMA on hypoxic vasoconstriction. Orienting experiments demonstrated that PMA in a dose of 10^{-9} M increased hypoxic vasoconstriction and that 5×10^{-8} M potentiated the hypoxic vasoconstriction to a degree comparable to that of vanadate (3×10^{-5} M) (figs. 4 and 5). PMA (5×10^{-8} M) occasionally caused a small rise in pulmonary base-line perfusion pressure. Addition of staurosporine (1 μg/ml) to the PSS perfusate before PMA or vanadate prevented the potentiation induced by either PMA (5×10^{-8} M) or vanadate (3×10^{-5} M) (figs. 4 and 5) (*i.e.*, the magnitude of hypoxic vasoconstriction was now similar to that before addition of the potentiating agent). As can be seen from figure 4, staurosporine reduced but did not abolish the vanadate-induced increase in basal perfusion pressure.

Effect of NCDC on PMA- and vanadate-induced potentiation of hypoxic vasoconstriction. NCDC, the PLC inhibitor, inhibited both the potentiation of hypoxic vasoconstriction due to PMA (5×10^{-8} M) and the potentiation of the hypoxic pressor response due to vanadate (figs. 4 and 5). NCDC, in this particular perfusate concentration (5×10^{-5} M), reduced the control hypoxic vasoconstriction (*i.e.*, without pretreatment by a potentiating agent) by 40% ($n = 4$).

Effect of nifedipine on PMA- and vanadate-induced potentiation of the hypoxic pressor response. In pilot experiments, we found that nifedipine (5×10^{-8} M) blocked approximately 80% of hypoxic vasoconstriction when added to the PSS, but only 10% of angiotensin II-induced vasoconstric-

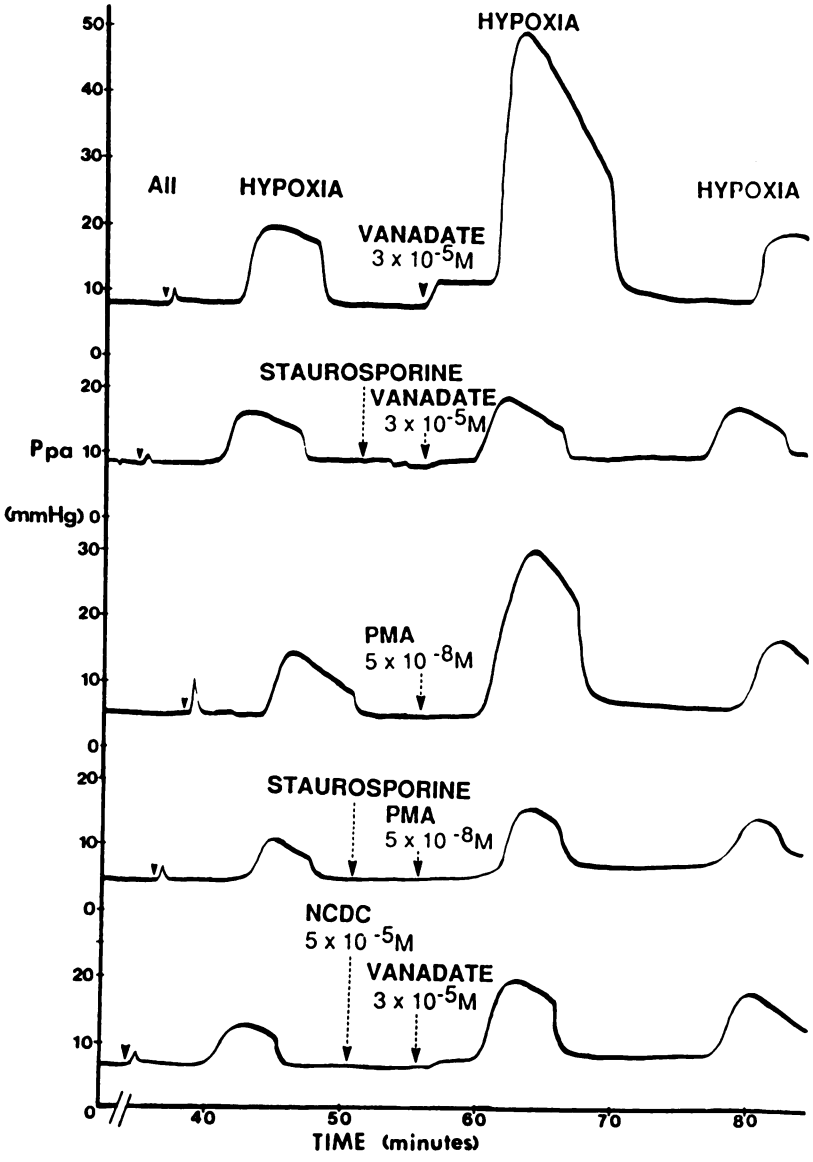

Fig. 4. Pulmonary artery pressure tracings from representative experiments in isolated rat lungs perfused with a physiological salt solution. Vanadate is added to the perfusate after two pairs of angiotensin II (AII)- and hypoxia-induced (FIO_2 0.00) pressor responses (only one pair is shown in the figure). The potentiation of hypoxic vasoconstriction after vanadate is transient (1st panel). The potentiation of hypoxic vasoconstriction after PMA (3rd panel) is transient. Staurosporine addition (1 μg/ml) prior to vanadate (2nd panel) or PMA (4th panel) abolishes the potentiation of hypoxic vasoconstriction. NCDC addition to the perfusate before vanadate (bottom panel) inhibits the potentiation of hypoxic vasoconstriction.

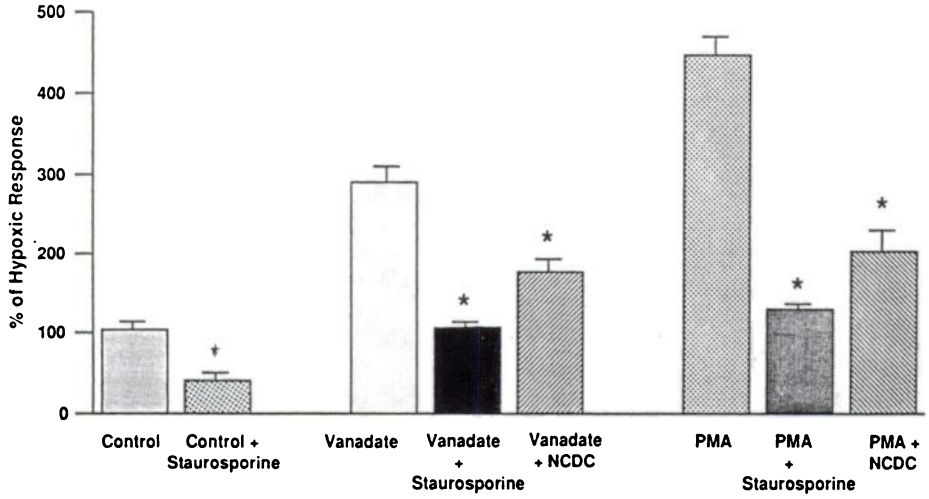

Fig. 5. Effect of vanadate (3×10^{-5} M) and PMA (5×10^{-8} M) on hypoxic vasoconstriction; the data are expressed as percent of the hypoxic vasoconstriction before the addition of either PMA or vanadate. The potentiation of hypoxic vasoconstriction by vanadate or PMA is prevented by staurosporine (1 μg/ml) ($n = 4$ each) and NCDC (5×10^{-5} M) ($n = 4$). $^{+}P < .05$ comparison with control hypoxic response. $^{*}P < .05$ comparison between vanadate and vanadate + staurosporine, or PMA and PMA + staurosporine, or vanadate and vanadate + NCDC, or PMA and PMA + NCDC.

tion. This dose of nifedipine (which blocked hypoxic vasoconstriction) reduced the pressor response caused by vanadate plus hypoxia or plus PMA (fig. 6). However, the pressor response due to vanadate plus hypoxia after nifedipine was still greater than the usual hypoxic response in control lungs (fig. 7). Similar results were obtained when PMA (5×10^{-8} M) was used instead of vanadate, indicating perhaps (within the limitation of the specificity of nifedipine as an inhibitor of Ca^{++} entry) that the potentiation of hypoxic vasoconstriction was, in part, Ca^{++} flux-independent. Also of interest, nifedipine (5×10^{-8} M) had little effect on vanadate-induced pulmonary perfusion pressure increase (fig. 6).

Discussion

In this study we show that sodium orthovanadate greatly increases the magnitude of hypoxic vasoconstriction. Because this potentiation occurred in lungs that were perfused with blood, as well as in lungs perfused with cell-free PSS, the effect of vanadate appears not to be mediated by formed blood elements or plasma factors. Although vanadate has some vasoconstricting effect of its own, which manifested itself in most of the lungs as an increase in base-line perfusion pressure, vanadate increased the pulmonary vasoconstriction to hypoxia more than that to the pressor agents angiotensin II and KCl. This indicates that vanadate had affected hypoxic vasoconstriction preferentially. Of further importance is our finding that vanadate shifted the dose-response curve for oxygen, thus allowing hypoxic vasoconstriction at astonishingly high oxygen concentrations. Vanadate also potentiated the vasoconstriction due to the hypoxia mimic, cyanide. The potentiation of hypoxic vasoconstriction was independent of cyclooxygenase-derived arachidonic acid metabolites (data not shown), not affected by oxygen radical scavengers, but prevented by an inhibitor of PLC and an inhibitor of PKC, and only partially reduced by nifedipine. Thus, the mechanism of potentiation of hypoxic vasoconstriction by vanadate depends to some degree on the activation of a PKC.

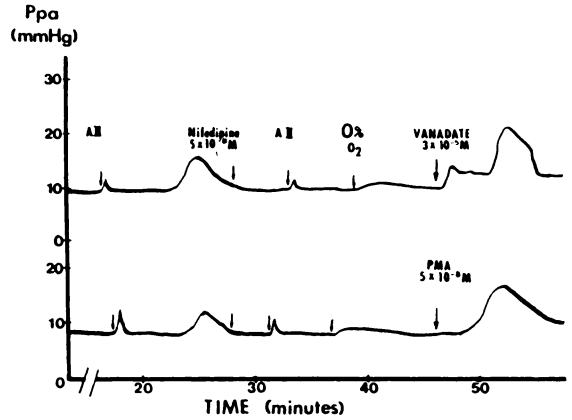

Fig. 6. Pulmonary artery pressure tracings from representative experiments. Addition of nifedipine subsequent to the reference hypoxic pressor response. Nifedipine blocks subsequent hypoxic vasoconstriction more than the vasoconstriction to angiotensin II (0.1 μg bolus injection). Thereafter, the base-line pressure increase after vanadate is preserved. Nifedipine does not block hypoxic pressor responses in the presence of vanadate (above) or PMA (below).

The effect of vanadate on hypoxic vasoconstriction, which we demonstrate in the current experiments is indeed impressive, and goes beyond the reversal of the vascular hyporeactivity of a salt solution-perfused lung preparation (*e.g.*, McMurtry *et al.*, 1976). The magnitude of hypoxic vasoconstriction in the presence of vanadate depends on the circulating vanadate concentration and on the FIO_2. Yet, the hypoxic pressor response in lungs perfused with a salt solution which contains 3×10^{-5} M vanadate, challenged with hypoxia, is larger than that commonly observed in control lungs perfused with blood. Whereas several other agents [including 4-aminopyridine (McMurtry *et al.*, 1976) and phorbolesters (Orton *et al.*, 1990; Raffestin and McMurtry, 1987)] have been shown to potentiate hypoxic vasoconstriction in salt solution-perfused rat lungs, vanadate also increases the oxygen sensitivity of the lung. This increase in oxygen sensitivity of the lung by vanadate again cannot be explained by the reversal of a general vascular hyporeactivity of the salt solution-perfused lungs because all the lungs showed strong vasoconstriction to hypoxia before vanadate administration. Our interpretation of the vanadate effects on vascular reactivity of the lung is that vanadate had affected both the oxygen-sensing mechanism as well as the contractile machinery of the vascular smooth muscle cells. In support of the latter is that vanadate enhanced vasoconstriction in our studies after angiotensin II and the report of others that demonstrated enhanced tension after vanadate in isolated rabbit aorta and portal vein preparations (Fox *et al.*, 1983; Shimada *et al.*, 1986; Sunano *et al.*, 1988). In support of the former is the data of this present study which demonstrate that salt solution-perfused

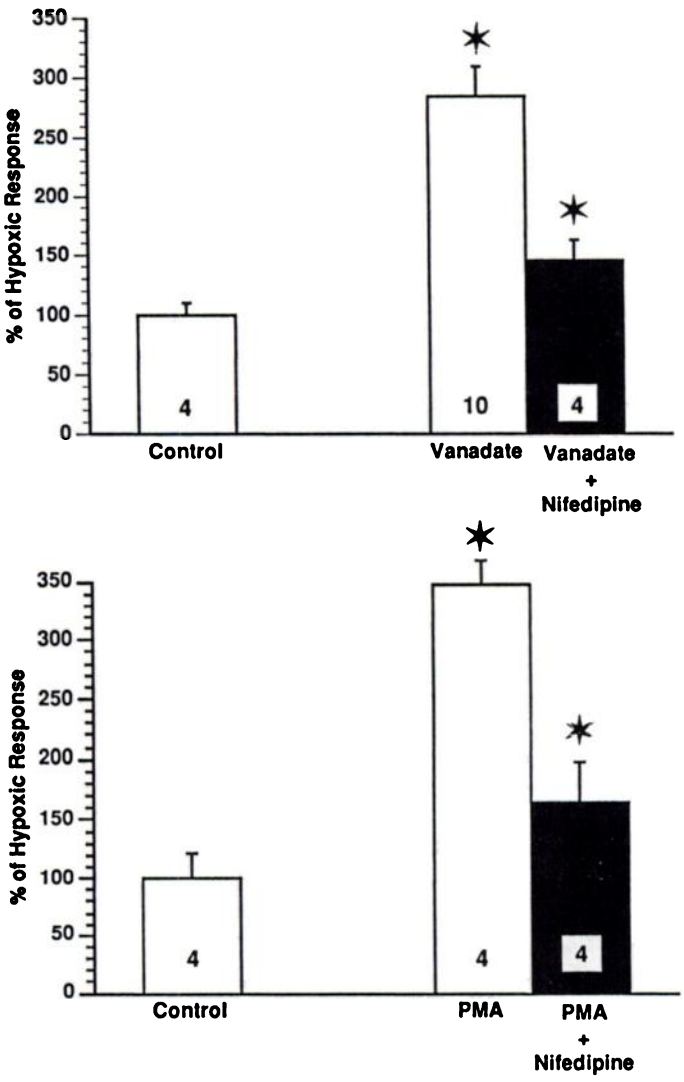

Fig. 7. Effect of nifedipine on vanadate- or PMA-potentiated hypoxic vasoconstriction. As in figure 5, data are presented as percent of the preceding hypoxic pressor response. The lungs that received either vanadate (3×10^{-5} M) or PMA (3×10^{-8} M) were different from those which received either nifedipine and vanadate or nifedipine and PMA. *P < .05, comparison is made with the control responses.

lungs can be made to constrict when challenged with 15% oxygen (which results in a pulmonary venous effluent PO_2 of 100 torr), thus greatly enhancing the oxygen sensitivity of the lung. That vanadate can alter oxygen sensing has previously not been appreciated, although vanadate has been reported to enhance oxygen binding to bovine hemoglobin and myoglobin (Sakurai *et al.*, 1982) and vanadium deficiency has been shown to increase the hematocrit in rats (Nechay, 1984). It has been long recognized that cyanide can mimic hypoxia in that it stimulates ventilation and causes pulmonary vasoconstriction, perhaps by affecting oxygen sensing (the cyanide stimulus is "perceived" by the sensor like a drop in PO_2). In this regard, it is interesting that vanadate potentiated not only hypoxic vasoconstriction, but also that due to the hypoxia mimic cyanide.

It is apparent that our attempts to isolate the mechanism of vanadate-induced potentiation of the hypoxic pressor response are in serious jeopardy because of the many known cellular actions of vanadate. As pointed out above, vanadate alters the activity of membrane-bound enzymes [ATPases, reduced nicotinamide adenine nucleotide oxidase, adenylate cyclase (Cantley *et al.*, 1978; Keller *et al.*, 1989; Markus *et al.*, 1989; O'Neal *et al.*, 1979; Ramasarma and Crane, 1981; Varecka and Carafoli, 1982; Xie *et al.*, 1989)] and likely affects guanine nucleotide binding proteins (G proteins) (Kanaho *et al.*, 1988) and the ATP-dependent transport of glutathione-*s*-conjugates like leukotriene C_4 (Ishikawa, 1989). In the present studies, we were able to rule out a participation of cyclooxygenase-derived arachidonic acid metabolites in the potentiation of hypoxic vasoconstriction by vanadate because the vanadate effect persisted in the presence of cyclooxygenase-blocking doses of meclofenamate (data not shown). We were also able to rule out a critical participation of oxygen radicals in the vanadate-potentiated hypoxic pressor response because pretreatment of lungs with very high concentrations of SOD in combination with catalase was without influence. Our finding that catechol, norepinephrine and ascorbate diminished the vanadate effect are likely due to binding of vanadate and reduction of VO_3^- to VO^{++} (Nechay, 1984) rather than due to oxygen radical scavenging (data not shown).

Although impressed by the multiplicity of cellular vanadate actions we felt encouraged by recent reports which demonstrated an intriguing similarity between vanadate and phorbol ester actions. Montesano and coworkers (Montesano *et al.*, 1988) showed a comparable *in vitro* induction of angiogenesis by vanadate (10^{-5} M) and by PMA, and argued that increased protein phosphorylation might be the common mechanism of vanadate and PMA action. They indeed demonstrated in cultured endothelial cells an increase in ^{32}P-labeled phosphotyrosine after either PMA or vanadate addition (Montesano *et al.*, 1988). Based on this and on an earlier report of altered tyrosine-specific protein phosphorylation by vanadate (Macara, 1986), we compared the actions of PMA and vanadate on hypoxic vasoconstriction, and used a purportedly specific inhibitor of PKC, staurosporine (Tamaoki *et al.*, 1986), in concentrations which were low enough not to abolish pulmonary vascular reactivity. We reasoned further that, if PKC was involved in vanadate- as well as PMA-induced potentiation of hypoxic vasoconstriction, a different drug, an inhibitor of PLC, might also inhibit the vanadate-induced potentiation of hypoxic vasoconstriction. Indeed, our findings taken together are consistent with a role of PLC and PKC activation in the vanadate-potentiated hypoxic pressor response. First, the PMA- and vanadate-induced potentiations of the hypoxic pressor responses were transient and similar in magnitude. Second, the potentiation of hypoxic vasoconstriction by vanadate or PMA was abolished by staurosporine (1 μg/ml). Third, the PLC inhibitor NCDC inhibited both vanadate and PMA-induced potentiation of hypoxic vasoconstriction. And fourth, the potentiation of hypoxic vasoconstriction (rather than hypoxic vasoconstriction itself) was not crucially dependent on calcium influx, because the potentiation was not abolished by nifedipine. Activation of PKC leads to increased intracellular calcium concentration (Macara, 1986), but does not depend critically on calcium entry (Exton, 1988; Nishizuka, 1986). Thus, with full recognition of the potential limits of the specificity of the inhibitors used in these experiments, we conclude that vanadate-induced potentiation of hypoxic vasoconstriction involves the activation of a PLC and of PKC. An alternative explanation of our data (because our interpretation is based on data obtained by using inhibitors) would be that staurosporine and NCDC affect intracellular calcium levels directly by some un-

known mechanisms. However, it is unlikely that the dose of staurosporine used by us prevented the rise of intracellular calcium in the lungs that one would expect to occur after vanadate or PMA (Macara, 1986; Nishizuka, 1986; Sunano *et al.*, 1988), because we observed recently that this dose of staurosporine (1 μg/ml) did not inhibit the calcium-dependent release of leukotrienes from salt solution-perfused rat lungs (Voelkel *et al.*, in press). Nifedipine blocked 80% of hypoxic vasoconstriction, consistent with earlier reports in which the less specific Ca^{++} blocker verapamil had been used (McMurtry *et al.*, 1976), but the combination of nifedipine and vanadate resulted in a hypoxic pressor response that was larger than that observed in the lungs before vanadate addition. Therefore (to the degree that nifedipine blocks specifically voltage-dependent Ca^{++} channels), and in contrast with hypoxic vasoconstriction *per se*, the potentiation of hypoxic vasoconstriction by vanadate appears to be, in part, independent of Ca^{++} influx.

We conclude that vanadate, known for its multiplicity of cellular actions, potentiates hypoxic vasoconstriction in part by increasing the oxygen sensitivity of the lungs. It appears, based on indirect evidence, that vanadate exerts at least some of its effect by activating the PLC/PKC axis, and we speculate that vanadate alters both the lung sensor and the effector that interact when the decrease in alveolar oxygen tension leads to precapillary arteriolar constriction.

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