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Influence of vanadate on glycolysis, intracellular sodium, and pH in perfused rat hearts

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Abstract

Vanadium compounds have been shown to cause a variety of biological and metabolic effects including inhibition of certain enzymes, alteration of contractile function, and as an insulin like regulator of glucose metabolism. However, the influence of vanadium on metabolic and ionic changes in hearts remains to be understood. In this study we have examined the influence of vanadate on glucose metabolism and sodium transport in isolated perfused rat hearts. Hearts were perfused with 10 mM glucose and varying vanadate concentrations (0.7–100 μM) while changes in high energy phosphates (ATP and phosphocreatine (PCr)), intracellular pH, and intracellular sodium were monitored using ^{31}P and ^{23}Na NMR spectroscopy. Tissue lactate, glycogen, and (Na^+, K^+)-ATPase activity were also measured using biochemical assays. Under baseline conditions, vanadate increased tissue glycogen levels two fold and reduced (Na^+, K^+)-ATPase activity. Significant decreases in ATP and PCr were observed in the presence of vanadate, with little change in intracellular pH. These changes under baseline conditions were less severe when the hearts were perfused with glucose, palmitate and β-hydroxybutyrate. During ischemia vanadate did not limit the rise in intracellular sodium, but slowed sodium recovery on reperfusion. The presence of vanadate during ischemia resulted in attenuation of acidosis, and reduced lactate accumulation. Reperfusion in the presence of vanadate resulted in a slower ATP recovery, while intracellular pH and PCr recovery was not affected. These results indicate that vanadate alters glucose utilization and (Na^+, K^+)-ATPase activity and thereby influences the response of the myocardium to an ischemic insult. (Mol Cell Biochem **170:** 53–64, 1997)

Key words: vanadate effects, glycolysis, intracellular sodium, NMR, perfused rat hearts, ischemia-reperfusion

Introduction

Interest in the biological importance of vanadium compounds and of their role in human metabolism has recently increased due to their potential usefulness in the management of diabetes [1]. Early studies have demonstrated that vanadium is essential in ensuring normal growth in chicks and rats [2–5]. Vanadate (+ 5 oxidation state) and/or vanadyl (+ 4 oxidation state) ions have been shown to inhibit (Na^+, K^+)-ATPase *in vitro* [6, 7], influence the activities of various enzymes [2,4] and exert a number of insulomimetic actions in a variety of cell types [8]. Studies, *in vitro*, have demonstrated a multitude of metabolic effects by vanadium compounds [9], such as (a) enhanced glucose uptake and oxidation in adipocytes [10–12], (b) increased glycogen synthesis in adipocytes and liver [9, 13, 14], and (c) inhibition of glycolysis in certain cell types [15]. In muscle, vanadium and insulin produce qualitatively similar changes in glucose metabolism, but the mag-

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nitude of these may differ depending on the metabolic pathway [1, 16]. Inhibition of hepatic cholesterol and phospholipid synthesis by vanadium compounds have also been reported [17, 18]. The mechanism of the insulin like action of vanadium compounds *in vivo* is still being investigated in detail [14], much attention being focussed on its effects on carbohydrate metabolism [19–21]. Recent studies have demonstrated that oral administration of vanadium compounds normalizes blood glucose levels, cardiac performance, and fluid intake in diabetic rats [1, 22–24]. The *in vitro* and animal studies have been recently followed by clinical studies of administration of small doses of vanadyl sulphate or sodium metavanadate to diabetic patients for short periods of time. Although these doses are about 100-fold lower than those used in most animal studies, beneficial effects were observed [25, 26].

Vanadate has been shown to exert a positive inotropic effect on the cardiac muscle of some animal species [27–29], whereas in other species, a negative inotropic effect is observed at lower concentrations [28, 29]. Although vanadate inhibits cardiac (Na^+, K^+)-ATPase [4, 30] and stimulates adenylyl cyclase increasing tissue cyclic AMP [28, 31], its inotropic effect does not appear to involve either of these mechanisms [28, 29, 32]. The influence of vanadate on myocardial Ca^{2+} transport has been suggested to be due to the inhibition of sarcolemmal (SL) and sarcoplasmic reticular (SR) Ca^{2+}-stimulated ATPase activity by vanadate [33–36]. Insulin-like effects of vanadate on the myocardium [14, 19, 37] appear to proceed by mechanisms different from those of insulin [38].

Since the above actions of vanadate can profoundly influence high energy phosphate metabolism, sodium and calcium homeostasis, we hypothesized that vanadate perfusion would increase glycogen synthesis, inhibit glycolysis as well as the (Na^+, K^+)-ATPase activity, and influence the metabolic and ionic changes during ischemia and reperfusion. Therefore, we measured changes in high energy phosphates and intracellular pH by ^{31}P NMR and intracellular sodium by ^{23}Na NMR using $TmDOTP^{5-}$ as a shift agent [39–41]. These NMR results were complemented by biochemical assays on the effect of vanadate on heart glycogen, lactate and (Na^+, K^+)-ATPase activity under normal and ischemic conditions. Simultaneous observation of all the vanadate effects on the perfused rat heart model allowed further understanding of the molecular mechanisms of its action.

Materials and methods

All experiments were performed with the approval of the Animal Use and Care Committee for Research at the University of Texas at Dallas and the University of California, Davis.

Materials

All chemicals and biochemicals were obtained from Sigma Chem. Co., unless stated otherwise. The macrocyclic ligand 1, 4, 7, 10-tetraazacyclododecane -N, N′, N″, N‴-tetra (methyenephosphonate) ($DOTP^{8-}$) and its thulium (III) complex ($TmDOTP^{5-}$) were synthesized and characterized as described elsewhere [39–41]. Male Sprague-Dawley rats (250–300 g) with free access to food and water were anesthetized with ether and injected in the femoral vein with 200 IU heparin before excision of the heart, which was then placed in 4°C perfusion medium. The aorta was immediately cannulated and the heart was perfused in the Langendorff mode at a perfusion pressure of 70 cm H_2O [40]. A latex balloon was placed in the left ventricle. Developed pressure and spontaneous heart rate were monitored using a fluid-filled catheter and recorded on a Coulbourne strip chart recorder. The heart was placed in an 18 mm o.d. NMR tube and perfused as described elsewhere [40]. The recirculating perfusate was a phosphate-free Krebs-Henseleit (K-H) solution containing (in mM) 143 Na^+, 130.8 Cl^-, 25 HCO_3^-, 5.0 K^+, 3.9 Ca^{2+}, 1.2 Mg^{2+}, 1.2 SO_4^{2-}, 3.5 $TmDOTP^{5-}$ and 10 glucose continuously bubbled with a mixture of 95% O_2 and 5% CO_2. Small aliquots of a 50 mM ammonium metavanadate (from Aldrich Chem. Co.) aqueous solution were added to the perfusate in order to obtain the desired final concentration of vanadate.

NMR spectroscopy

^{23}Na and ^{31}P NMR spectra were obtained on a General Electric GN 500 (11.75 T) spectrometer operating at 132.28 MHz for ^{23}Na and at 202.415 MHz for ^{31}P, equipped with an 18 mm probe tunable to ^{23}Na or ^{31}P, using conditions described elsewhere [40]. Magnetic field homogeneity was adjusted using the ^{23}Na free induction decay; a linewidth of 24–26 Hz on the extracellular ^{23}Na resonance was usually obtained in the presence of 3.5 mM $TmDOTP^{5-}$ in the perfusate. ^{23}Na spectra were acquired using a ± 1600 Hz spectral width, 2048 data points, a 90°pulse, 184 acquisitions and a 0.33 sec interpulse delay over a period of 1 min. Control ^{31}P spectra before and after the addition of $TmDOTP^{5-}$ and serial ^{31}P spectra after the addition of vanadate were acquired over a ± 4000 Hz spectral width using 4096 data points in 4 min. with a 40° pulse, 200 acquisitions and a 1.2 sec interpulse delay. For the ischemia and recovery experiments, ^{31}P and ^{23}Na NMR measurements were made on control hearts (n = 3) and 40 µM vanadate treated hearts (n = 3) in the presence of 3.5 mM $TmDOTP^{5-}$ and $[Ca^{2+}]_t$ = 3.9 mM during ischemia periods of 12 min (^{31}P experiments) or 10 min (^{23}Na experiments) and during recovery for 15 min (^{31}P experiments) or 10 min (^{23}Na experiments). The NMR spectra were collected every 3 min (^{31}P spectra) or every minute (^{23}Na spectra). ^{23}Na and ^{31}P spec-

tra were processed with a combined exponential and Gaussian function of 6 Hz and 40 Hz, respectively [40].

^{23}Na NMR spectra were analysed using the least-squares fitting algorithm supplied in the General Electric software, which allowed the calculation of linewidth, peak height, area and chemical shift for each spectrum [40]. Intracellular Na^+ concentrations were calculated from peak areas as previously described [40]. Intracellular pH values were obtained in the usual manner from the chemical shift of the P_i resonance in the ^{31}P NMR spectra [40]. Intracellular Na^+ concentrations and pH are reported as means. Standard deviations (SD) are very similar to those obtained in previous studies [40]. In particular, SD values for all pH determinations were lower than 0.012.

Lactate measurements

Hearts were freeze-clamped at different time points (prior to ischemia, end of ischemia, and end of reperfusion, n = 7 hearts per time point, see conditions of the NMR experiments) and the lactate extracted with 6% perchloric acid. The perchloric acid extracts were then neutralized with KOH and the lactate measured using standard biochemical assays [42].

Glycogen measurements

Glycogen content prior to ischemia was measured in freeze-clamped hearts. Following perfusion with or without vanadate the hearts were rapidly freeze-clamped using aluminium tongs and assayed for glycogen [43]. Glycogen content is expressed as µmol glucose equivalents per gram dry weight (µmol glu/gdw).

Measurements of (Na+, K+)-ATPase activity

Ouabain sensitive (Na+, K+)-ATPase activity was measured in homogenates of hearts (n = 6) and expressed as µM ADP formed/min/mg total protein [44]. Heart tissue was homogenized at 4°C in 2 ml of 0.2 M sucrose-0.02 M Tris-HCl buffer (pH 7.5) containing 100 µl of 57 mM phenylmethylsulfonyl fluoride and 10 µl of 1 mg/ml leupeptide as protease inhibitor, with a polytron homogenizer for four periods, each period not exceeding 15 sec, and then centrifuged at 100 g for 10 min at 4°C. The reaction was started by the addition of 20 µl of supernatant to a 1 ml × 1 cm cuvette containing final concentrations of 100 mM NaCl, 10 mM KCl, 2.5 mM $MgCl_2$, 2 mM EGTA, 1 mM Tris-ATP, 1 mM phosphoenolpyruvate, 30 mM imidazole-HCI buffer at pH 7.4, 0.15 mM NADH, 50 µg lactate dehydrogenase, 30 µg pyruvate kinase with or without ouabain. After a 30 min stabilization period, the linear rate of oxidation of NADH was monitored at 340 nm. ATPase activity was calculated from the linear portion of the curve using the mM extinction coefficient of NADH, volume of the reaction mixture, and the amount of heart homogenate, as shown below:

ATPase activity = {slope(OD units/min)/6.22(OD units.ml/µmol)} × {1 ml/protein in mg}

The protein concentrations were assayed in each freeze-clamped heart.

Protocols

NMR experiments and biochemical assays were performed in which some hearts were subjected to baseline perfusion with vanadate while other hearts were subjected to ischemia and reperfusion in the presence of vanadate.

Group I: perfusion with vanadate under baseline conditions

Glucose as a substrate

Hearts (n = 3) were subjected to 20 min of perfusion in normal Krebs-Hensleit buffer (10 mM glucose as the sole substrate) followed by increasing concentrations of vanadate (from 0.7–100 mM at least 10 min at each concentration). In the cases of 40 mM and 100 mM concentrations the hearts were exposed for a longer period to determine the extent of metabolite and functional deterioration with time.

Mixed substrate

Hearts (n = 3) were subjected to 20 min of perfusion in Krebs-Hensleit buffer containing 10 mM glucose, 1 mM palmitate, and 0.25 mM β-hydroxybutyrate followed by increasing concentrations of vanadate (from 20–40 µM) to determine the extent of metabolite and functional deterioration with time.

Group II: presence of vanadate during ischemia and reperfusion

Set A

Six hearts were perfused with vanadate-free buffer then subjected to global ischemia followed by reperfusion in the same buffer.

Set B

Six hearts were perfused with 40 mM vanadate prior to ischemia, then subjected to global ischemia followed by reperfusion with 40 mM vanadate.

Set C
Six hearts were perfused with 40 mM vanadate prior to ischemia, then subjected to global ischemia followed by reperfusion in vanadate-free perfusate.

In the ^{23}Na NMR experiments the duration of global ischemia was 10 min followed by 10 min of reperfusion, while in ^{31}P NMR experiments duration of global ischemia was 12 min followed by 15 min of reperfusion.

Statistical analysis

Results are given as the mean ± SD for the indicated number of rat hearts. Comparisons among control and vanadate-treated hearts were carried out by comparing means using Student *t* test. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Effect of vanadate on heart developed pressure

The left ventricular pressure (LVDP) decreased as function of perfusate vanadate concentration. The initial positive inotropic response observed with 1 µM vanadate diminished as the vanadate concentration was increased to 10 µM. At vanadate concentrations of 10 µM and higher, a negative inotropic response was observed. The percent reduction in LVDP upon treatment with 10, 20, 40 µM vanadate concentrations for 10 min was 44 ± 6%, 59 ± 7%, and 63 ± 5%, respectively. Continuous perfusion with 40 µM vanadate for 26 min or more resulted in complete loss of LVDP and heart rhythm. Washout of vanadate with normal Krebs-Hensleit buffer resulted in a marginal recovery of LVDP function (27 ± 6%) and heart rhythm. These results were consistent with the effects of vanadate on contractile function as reported in the literature [2, 27–29, 32, 35, 36]. In particular, it has been shown that the inotropic effect of 10 mM vanadate is highly dependent on the Ca^{2+} content of the medium (eg 10 mM vanadate causes a LVDP increase of 459% at 0.3 mM Ca^{2+} but a decrease to 66% at 2.0–3.0 mM Ca^{2+} [36]).

Effect of vanadate under baseline conditions

High energy phosphates and intracellular pH
Serial ^{31}P NMR spectra (recorded every 4 min) obtained before and after successive additions of vanadate to the perfusion medium are shown in Figs 1A–C. As the vanadate concentration was increased, the ^{31}P NMR resonance intensities of phosphocreatine (PCr), β-ATP, and inorganic phosphate (P_i) changed dramatically (see Table 1). The successive reductions in PCr and ATP paralleled the increase in P_i. Perfusion with 40 µM vanadate for 26 min resulted in reduction of PCr by about 87% (compared to baseline) and of ATP by 91%.

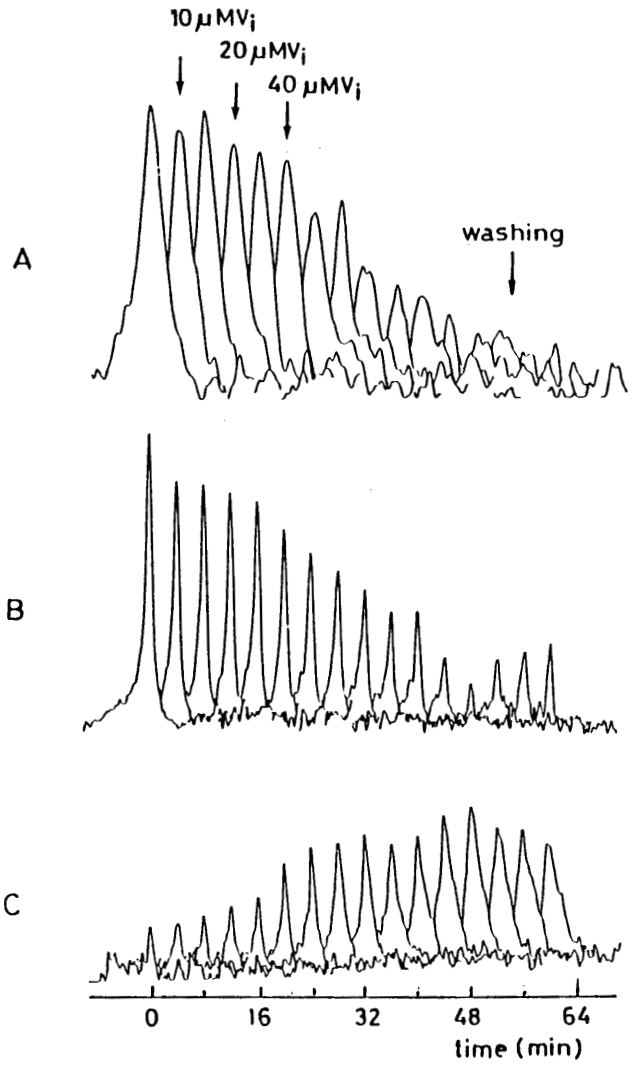

Fig. 1. Time evolution of the ^{31}P NMR signals (A, B, C) of a perfused rat heart, in the presence of increasing concentrations of vanadate and after its removal from the perfusion medium: (A) ATP P_β signal; (B) PCr signal; (C) P_i signal.

The changes in P_i were quite dramatic with increases of 5–6 times baseline control values during perfusion with 40 µM vanadate. The P_i chemical shift, however, was unchanged with vanadate, indicating that the intracellular pH had not changed. Therefore, under baseline perfused conditions we were unable to detect any marginal changes of pH due to vanadate, as the intracellular proton generating metabolites are constantly washed out in those conditions.

The observation of a broadening of the ^{31}P NMR resonances

Table 1. Changes in phosphocreatine (PCr), ATP, inorganic phosphate (P_i), and phosphomonoester (PME) expressed as a fraction of baseline, of perfused rat hearts under baseline conditions (n = 3), obtained by ^{31}P NMR, as a function of vanadate perfusion time

Hearts	Glucose substrate Time of exposure (min)			Mixed substrate Time of exposure (min)
	5	10	25	25
PCr				
control	1.00	0.99 ± 0.02	0.97 ± 0.03	0.99 ± 0.03
vanadate (20 μM)	0.82 ± 0.06	0.78 ± 0.04	nd	0.88 ± 0.04
vanadate (40 μM)	0.67 ± 0.04	0.62 ± 0.06	0.13 ± 0.02	0.66 ± 0.12
β-ATP				
control	1.00	0.98 ± 0.03	0.98 ± 0.05	0.98 ± 0.02
vanadate (20 μM)	0.95 ± 0.03	0.82 ± 0.02	nd	0.92 ± 0.03
vanadate (40 μM)	0.77 ± 0.02	0.63 ± 0.04	0.09 ± 0.03	0.56 ± 0.09
P_i				
control	1.00	1.04 ± 0.03	1.02 ± 0.04	1.08 ± 0.02
vanadate (20 μM)	1.09 ± 0.04	1.27 ± 0.06	nd	1.42 ± 0.07
vanadate (40 μM)	1.82 ± 0.06	2.18 ± 0.09	2.55 ± 0.12	1.82 ± 0.12
PME				
control	1.00	1.16 ± 0.03	1.09 ± 0.02	1.02 ± 0.03
vanadate (20 μM)	1.06 ± 0.03	1.12 ± 0.04	1.32 ± 0.12	1.16 ± 0.07
vanadate (40 μM)	1.14 ± 0.08	1.36 ± 0.07	1.72 ± 0.18	1.28 ± 0.11

nd – Not determined. Values are the mean ± SD (n = 3 hearts in each group).

of ATP and P_i, but not of PCr, after exposure to extracellular vanadate and even after removal of vanadate by washing (Fig. 1), indicated that the intracellular monovanadate was at least partially reduced to the paramagnetic vanadyl species, VO^{2+} [45–49]. This species is known to bind preferentially to the phosphate groups of intracellular ATP and P_i, resulting in a broadening of their ^{31}P NMR signals [50].

In general, under normoxic conditions ATP is generated mostly via oxidative phosphorylation while during ischemic conditions ATP is generated principally via glycolysis. The phosphomonoester (PME) resonance in the ^{31}P NMR spectrum is a good indicator of phosphorylated compounds, specifically the intermediates of glycolysis. Under baseline conditions we observed small changes in PME resonance in the presence of vanadate when glucose was used as the substrate. The PME resonance was significantly greater in 40 μM vanadate perfused hearts (Table 1) compared to the untreated control hearts. Despite statistical significance in PME values with 40 μM vanadate perfusion, the changes in PME do not correlate with changes in ATP and PCr. In addition, when the hearts were perfused with mixed substrates (fatty acids, glucose, and ketone bodies), presence of 40 μM vanadate did not increase PME levels. The presence of 40 μM vanadate reduced ATP and PCr levels by a greater amount in glucose perfused hearts compared to mixed substrate perfused hearts. These observations suggest that the vanadate induced reduction in baseline ATP and PCr levels may be multifactorial. Under baseline conditions, based on PME levels, it is reasonable to conclude that inhibition of glycolysis is a minor component that accounts for the observed vanadate induced reduction in ATP. Therefore it is likely that vanadate may influence the ATP generating enzymes in the TCA cycle and/or influence ATP utilization.

The vanadate induced changes in ATP, PCr, and P_i were less dramatic in hearts perfused with a mixed substrate containing palmitate, glucose, and β-hydroxybutyrate compared with glucose perfused vanadate hearts. However, the vanadate induced reductions in phosphorylated metabolites, in the presence of mixed substrates, were still significantly greater than in untreated control hearts ($p = 0.003$). These data suggest that under baseline conditions vanadate influences high energy phosphate levels either by influencing their production (inhibiting ATP generating steps in oxidative phosphorylation) and/or utilization.

Glycogen

Perfusion with 40 μM vanadate significantly increased glycogen levels in hearts from 37.2 ± 3 μmol glu/gdw (n = 6) to 72.6 ± 11 μmol glu/gdw (n = 6). The magnitude of increase observed with vanadate perfusion was similar to that observed for hearts perfused with high levels of insulin (69.3 ± 7 μmol glu/gdw, n = 6, with 50 units liter^{-1} insulin). The precise mechanism by which vanadate increases glycogen is unclear. Recent studies by Liu and McNeill [51] demonstrated that vanadyl sulphate increased cAMP levels and reduced glycogen phosphorylase activity in diabetic hearts. Similar studies on the effect of vanadate on glycogen synthase, glycogen phosphorylase, and cAMP [28, 31] will help to understand the precise mechanism by which vanadate increases glycogen levels in perfused rat hearts.

(Na^+, K^+)-ATPase activity

Significant inhibition of (Na^+, K^+)-ATPase activity was observed at 40 μM and even at much lower concentrations of vanadate (0.7 μM) (see Table 2), in agreement with recent studies [36]. The activity of (Na^+, K^+)-ATPase can be lowered by direct inhibition or by lowering the availability of glycolytically produced ATP. Since vanadate can directly inhibit (Na^+, K^+)-ATPase as well as glycolysis, the magnitude of (Na^+, K^+)-ATPase inhibition is likely due to a cumulative effect of the above two mechanisms.

Intracellular sodium

In another series of experiments, the effect of vanadate addition on the intra- and extracellular sodium in hearts was investigated using ^{23}Na NMR spectroscopy. 3.5 mM $TmDOTP^{5-}$ was added to the perfusate together with excess (3.9 mM) Ca^{2+} to maintain free, ionized Ca^{2+}, $[Ca^{2+}]_f$, near 1.0 mM [39, 40]. ^{23}Na NMR spectra of hearts were collected before and

Table 2. Effect of vanadate on rat heart (Na^+, K^+)-ATPase activity measured in heart homogenates after perfusion under baseline conditions

Hearts	(Na^+, K^+)-ATPase activity (μmol/hour.mg total protein)
Control hearts	5.12 ± 0.06
Vanadate hearts (40 μM)	2.58 ± 0.03**
Vanadate hearts (0.7 μM)	3.12 ± 0.04**

**Significantly lower than in control hearts ($p = 0.005$). Values are the mean ± SD (n = 6 hearts in each group).

Table 3. Effect of vanadate on rat heart lactate measured in freeze-clamped heart extracts after perfusion under baseline, ischemic and reperfusion conditions

Hearts	Lactate (μmol/g dry weight)		
	Start	End ischemia	End reperfusion
Control	3.2 ± 0.2	176 ± 19	18.9 ± 2.7
Vanadate (40 μM)	2.2 ± 0.9	122 ± 11*	9 ± 1.2**

*,**Denotes significant differences between control and vanadate treated hearts, $p < 0.05$. Values are the mean ± SD (n = 7 in each group).

after addition of vanadate to the perfusion medium, in concentrations of 1, 10 and 40 μM. ^{31}P NMR spectra (conditions similar to that of ^{23}Na NMR experiments) during addition of vanadate, in the presence of $TmDOTP^{5-}$, showed no major alteration in intracellular pH or the intensities of the usual phosphorylated compounds. Despite the inhibition of (Na^+, K^+)-ATPase activity, no change in intracellular sodium concentration was observed in the presence of vanadate. The lack of changes in intracellular sodium could be due to the low visibility of intracellular sodium by NMR. It has been suggested that only about 40% of intracellular sodium is visible by NMR [39, 40]. To address this issue of sodium changes, we performed experiments measuring changes in intracellular sodium on freeze-clamped heart extracts using atomic absorption spectroscopy [39, 40]. This invasive technique is sensitive to changes in sodium, even in the nanomolar range, under baseline conditions. These experiments revealed that intracellular sodium was significantly higher in 40 μM vanadate perfused hearts compared to the controls (intracellular sodium being 41.2 ± 6.3 mM in vanadate hearts versus 24.6 ± 3.9 mM in controls, n = 6 in each group, $p = 0.03$).

Effect of vanadate during ischemia and reperfusion

LVDP before and after ischemia

The LVDP was 112.6 ± 9.2 cm of H_2O pressure in controls while it was 48 ± 5.4 cm of H_2O pressure in 40 μM vanadate perfused hearts prior to the start of global ischemia. On reperfusion, the LVDP recovery was 65.8 ± 11.4 cm of H_2O pressure in controls, 58.6 ± 14.2 cm of H_2O pressure in set B hearts (vanadate not present during reperfusion), and 78.9 ± 16.5 cm of H_2O pressure in set C hearts (vanadate present during reperfusion).

Lactate and intracellular pH

Levels of lactate were similar in control and vanadate hearts prior to ischemia. However, during ischemia and reperfusion, hearts exposed to vanadate had significantly reduced levels of lactate compared to the control hearts (Table 3). This demonstrated that glycolysis was inhibited by vanadate. The degree of intracellular acidification during ischemia (Fig. 2), as expected, was concordant with biochemical measurements of lactate release in the two groups. Intracellular pH was identical in the two groups immediately prior to global ischemia. During the first 5 min of global ischemia, the fall in pH was rapid in both control and vanadate hearts. After the first 5 min, the fall in pH continued in control hearts, but was blunted in vanadate hearts, resulting in significantly different end-ischemic pH values of 5.88 ± 0.12 and 6.46 ± 0.09 in control and vanadate hearts. Similar observations of reduced acidosis during global ischemia have been reported in glycolysis inhibited substrate-free pyruvate perfused hearts [52]. Upon reperfusion, both groups of hearts had similar rates of pH recovery. These observations show that the pH recovery mechanisms, such as the Na^+-H^+ exchanger, the $Cl^-HCO_3^-$ transporter, the lactate-H^+ cotransporter, are unaffected by vanadate.

Proton and lactate accumulation during ischemia may primarily be detrimental to the cardiocyte. Reperfusion after moderate periods of global ischemia can result in additional injury. Evidence in the literature suggests that the following sequence of events leads to irreversible myocyte injury: (a) proton production results in intracellular sodium accumulation (via the Na^+-H^+ exchanger) and (b) increases in sodium lead to calcium accumulation (via the Na^+-Ca^{2+} exchanger) [53]. However, recent findings have demonstrated that the mechanisms can operate whereby the intracellular sodium accumulation can be attenuated without limiting acidosis [54, 55].

Among the multiple potential sources of hydrogen ions in ischemic tissue [52], it has been shown that the hydrolysis of ATP is the main proton producing process during total global ischemia in the perfused rat heart [56, 57]. The levels of ATP are determined by the extent of glycogenolysis and glycolysis [56, 57]. Thus, the reduced acidosis observed during ischemia in the presence of vanadate correlates well with reduced glycogenolysis and glycolysis in the ischemic heart in the presence of vanadate.

High energy phosphates

Figures 3A and 3B demonstrate the changes in high energy phosphates observed in these three groups of hearts during ischemia and reperfusion. Vanadate exposure significantly lowered phosphocreatine (PCr) levels prior to initiation of

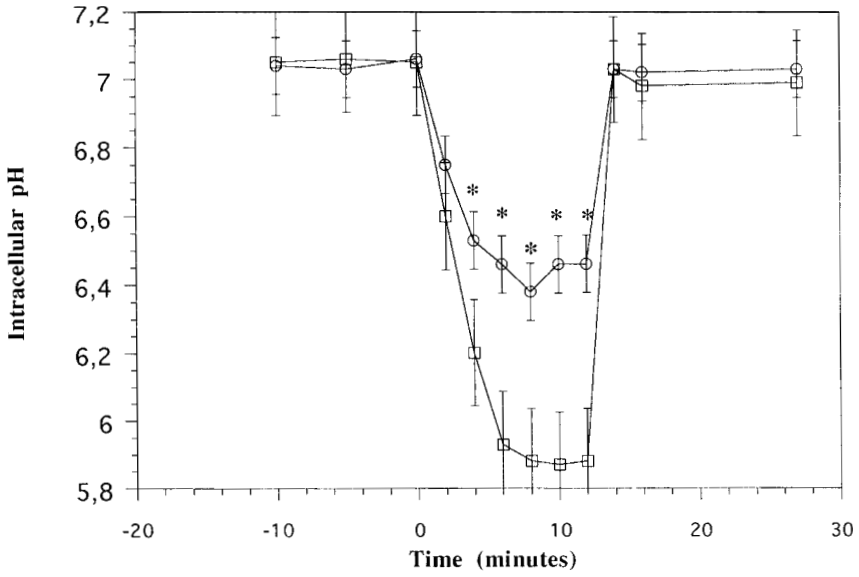

Fig. 2. Changes in intracellular pH, measured by ^{31}P NMR, of a perfused rat heart, during global ischemia and reperfusion. The symbols open squares and open circles represent control and 40 μM vanadate treated hearts, respectively. *Denotes that pH is significantly different from the control hearts ($p < 0.05$). At the time of –6 min hearts were exposed to 40 μM vanadate (vanadate hearts only), at the time of 0 ischemia was initiated, and the end of 12 min reperfusion was started.

global ischemia. PCr fell rapidly in the control group, while in vanadate hearts the loss of PCr was slower with complete depletion of PCr in all groups after the first five minutes of global ischemia. Reperfusion caused a rapid but partial return of PCr in all groups within 5 min.

Prior to global ischemia, the ATP levels were significantly reduced ($p < 0.05$) in vanadate hearts compared to the control hearts. During the first 5 min of ischemia, the level of ATP was maintained in both control and vanadate hearts. All hearts had negligible amounts of ATP remaining by 12 min of ischemia. From this data, it is not possible to distinguish the source of ATP (ie ATP from glycolysis or glycogenolysis) that contributes to proton production during ischemia.

In both the control and group B hearts (vanadate during ischemia alone, not upon reperfusion) ATP recovery was similar, with approximately 40% ATP recovery on reperfusion. When vanadate was present during reperfusion (set C hearts), the ATP recovery was significantly reduced relative to control hearts. This reduction in ATP recovery is likely due to the effect of vanadate on ATP production (via oxidative phosphorylation and glycolysis) and/or ATP utilization.

PME

It has been demonstrated by Schaefer *et al.* [52] that the end-ischemic PME/P_i ratio can reveal qualitative information on glycolysis during global ischemia. The end-ischemic PME/P_i ratios were higher in 40 μM vanadate perfused hearts compared to the controls (PME/P_i ratios were 0.41 ± 0.06 in vanadate hearts compared to 0.26 ± 0.03 in controls). It is well established that anaerobic glycolysis is the dominant metabolic pathway during global ischemia in perfused hearts [52, 54–57]. The lactate and PME/P_i data shown here suggest that vanadate inhibits glycolysis during global ischemia.

Intracellular sodium

Figure 4 shows the effect of vanadate on the time evolution of the intracellular sodium signal, $[Na^+]_i$, during global (10 min) ischemia and reperfusion. The presence of 40 μM vanadate in the perfusion medium did not prevent an increase in $[Na^+]_i$ during ischemia. During reperfusion, the presence of vanadate significantly slowed the return of intracellular Na^+ back toward preischemic levels. Studies have demonstrated that the recovery of intracellular sodium during reperfusion, is in part, dependent on the (Na^+, K^+)-ATPase activity [53, 54]. The slow recovery of intracellular sodium on reperfusion, observed in vanadate treated hearts, is suggestive of the inhibition of (Na^+, K^+)-ATPase by vanadate, consistent with our observation under baseline conditions. This is consistent with the observations described above of the effects of vanadate on the (Na^+, K^+)-ATPase activity of perfused hearts under baseline conditions.

Reduced levels of ATP have been demonstrated to influence the ion regulatory mechanisms such as the (Na^+, K^+)-ATPase, Ca^{2+}-ATPase, and the ATP sensitive K^+ channels [57, 58]. Therefore, the retarded intracellular Na^+ recovery in vanadate hearts is consistent with the reduced levels of ATP. Despite the limitation of acidosis, the slower intracellular Na^+ recovery due to inhibition of the (Na^+, K^+)-ATPase activity may offset the beneficial effects of reduced proton load during global ischemia in vanadate hearts.

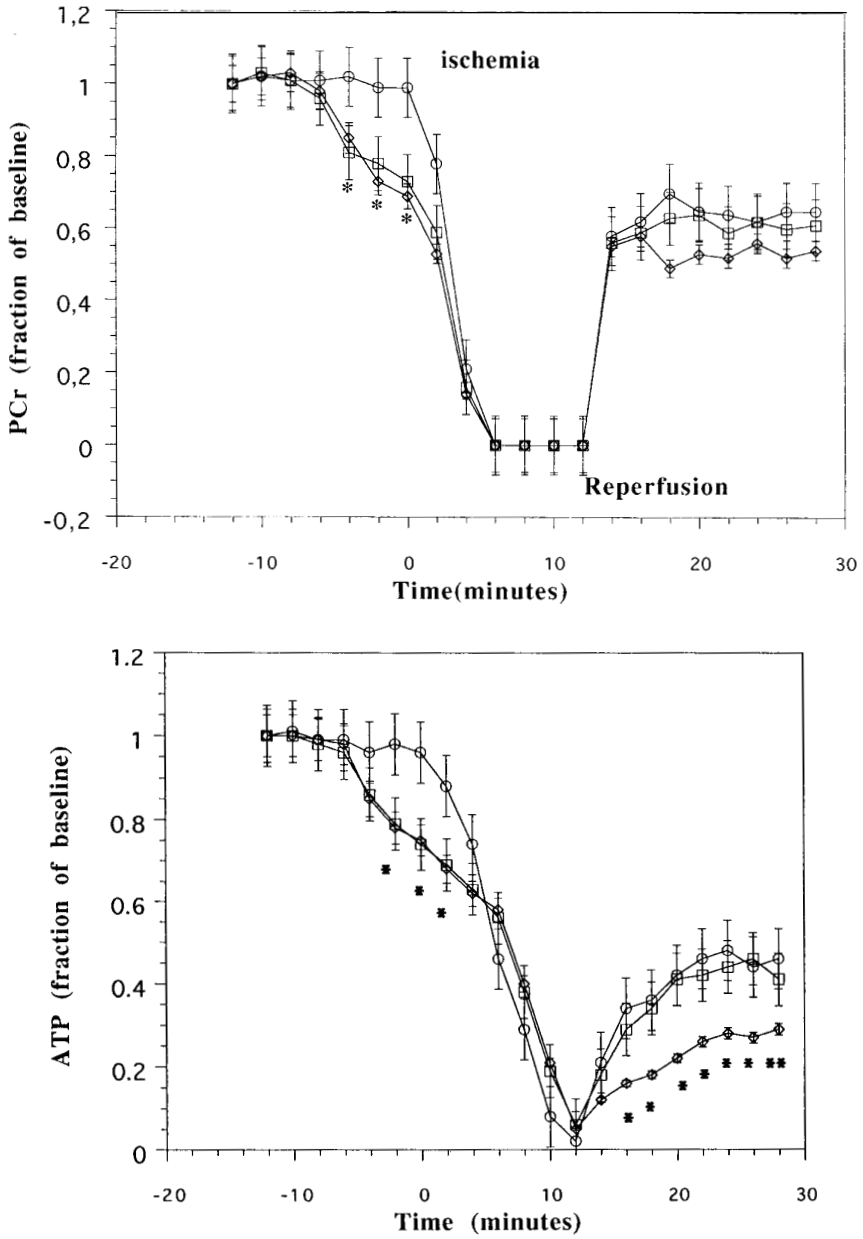

Fig. 3. Changes in PCr (3A) and ATP (3B), measured by ^{31}P NMR, of a perfused rat heart, during global ischemia and reperfusion. The control hearts are represented by open circles, the hearts treated with 40 µM vanadate only during ischemia are represented by open squares (set B), and the hearts treated with 40 µM vanadate during ischemia and reperfusion (set C) are represented by open diamonds. At the time of –6 min hearts were exposed to vanadate (vanadate hearts only), at the time of 0 ischemia was initiated, and at the end of 12 min reperfusion was started. ATP was significantly ($p < 0.05$) different during reperfusion in the set C hearts.

Limitations

The findings in this study should be interpreted within the limitations of the experimental design. First, the influence of vanadate on metabolites and ions, in the presence of physiological substrates (such as fatty acids, ketone bodies, etc.), would likely be less drastic than that obtained in this study with glucose as the only substrate. Second, the use of ^{23}Na NMR to measure intracellular sodium is restricted by the visibility of the intracellular sodium resonance. Typically, the visibility of intracellular sodium is about 40%. Therefore, despite lower activity of (Na^+, K^+)-ATPase, it was not possible to observe increases in intracellular sodium by NMR. However, independent intracellular sodium measurements

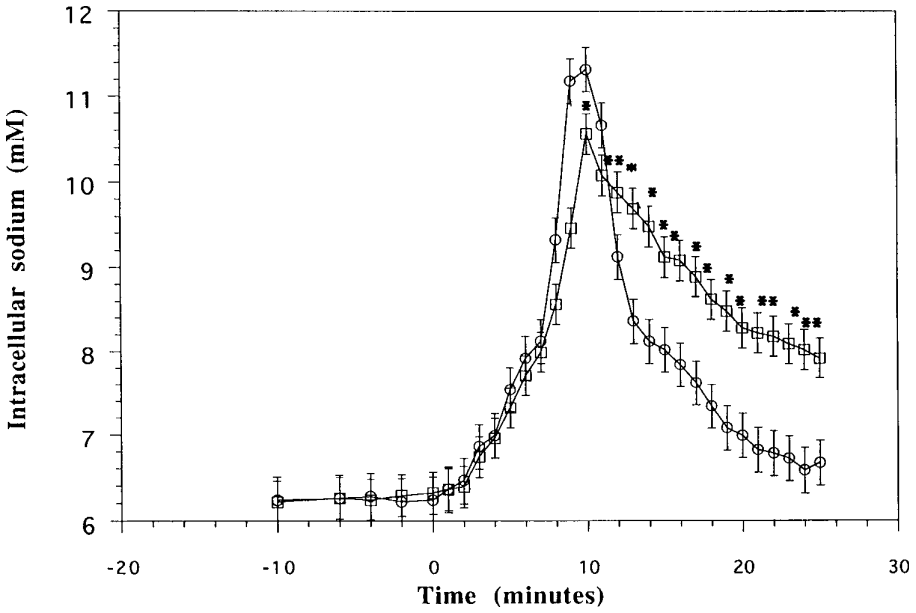

Fig. 4. Changes in intracellular Na^+ concentration, measured by ^{23}Na NMR, of a perfused rat heart, during (10 min) ischemia and reperfusion, in the absence (open circles) and presence (open squares) of 40 µM vanadate in the perfusion medium. At the time of –6 min hearts were exposed to vanadate, at the time of 0 ischemia was initiated, and the end of 10 min reperfusion was started. During the reperfusion period the intracellular sodium concentrations were significantly ($p < 0.05$) lower in vanadate hearts than in the control hearts.

using the atomic absorption technique revealed that vanadate increased intracellular sodium in hearts. Also, the *in vitro* (Na^+, K^+)-ATPase assay measurements were performed under saturating ATP conditions. Therefore, the activity measurements are more reflective of changes in enzyme sites per gram heart rather than ATP availability. Third, the results obtained in this study demonstrate the observed effects of vanadate but do not establish the mechanisms for these changes.

Conclusions

These data indicate that vanadate increased the glycogen synthesis, reduced the activity of (Na^+, K^+)-ATPase, and LVDP function of perfused rat hearts under baseline conditions. Vanadate limited acidosis and lactate accumulation during ischemia while impairing the recovery of intracellular sodium and ATP on reperfusion. These observations are consistent with our hypothesis that vanadate inhibits ATP generation and (Na^+, K^+)-ATPase, thereby influencing changes in ATP and intracellular sodium during ischemia and reperfusion in perfused rat hearts.

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