Vanadate Changes Ca^{2+} Influx Pathway Properties in Human Red Blood Cells

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Abstract. The properties of the basal Ca^{2+} influx (measured using cells labelled with $^{45}Ca^{2+}$) in intact human red blood cells (RBC) were compared with those of $^{45}Ca^{2+}$ influx induced by vanadate The basal Ca^{2+} influx was not sensitive to inhibitors of vanadate-induced Ca^{2+} influx such as the HS-reagent *p*-chloromercuribenzoate and low concentrations of Cu^{2+} and also the sensitivity to nifedipine was significantly weaker High K^+ known to suppress vanadate-induced $^{45}Ca^{2+}$ influx had no effect on the basal Ca^{2+} influx Both processes were saturated with Ca^{2+} but the latter was saturated at higher Ca^{2+} concentrations ($K_{M(Ca)}$ 2 1 vs 0 5 mmol/l) These experiments favour the notion that vanadate changes the properties of the inward-directed Ca^{2+}-transport pathway in human RBC membrane Vanadate-induced $^{45}Ca^{2+}$ influx was insensitive to pertussis toxin and cholera toxin, and several non-steroidal antiinflammatory agents did not influence it in a consistent manner Li^+ partly inhibited the $^{45}Ca^{2+}$ uptake Vanadate stimulated the incorporation of $^{32}P_{in}$ into PIP_2 in human but not in pig RBC which are known to be defective in the phosphoinositide metabolism and in the vanadate-induced $^{45}Ca^{2+}$ uptake These results suggest that the change in the Ca^{2+} influx pathway properties induced by vanadate may involve changes in the metabolism of phosphoinositides but not of the arachidonate metabolism nor G-protein activation

Key words: Red blood cells — Vanadate — Ca^{2+} influx

Abbreviations: EDTA – ethylenediaminetetraacetic acid, RBC – red blood cells TPP^+ – tetraphenylphosphonium K(Ca) – Ca^{2+}-activated K^+ channel, PA – phosphatidic acid, pCMB – para-chloromercuribenzoate, PI – phosphatidylinositol, PIP

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– phosphatidylinositol-4-phosphate, PIP_2 – phosphatidylinositol-4,5-bisphosphate, PTX – pertussis toxin, TTX – tetrodotoxin

Introduction

Vanadate has numerous pharmacological effects at both the cellular and the organism levels (for review see Nechay 1984, Rehder 1991) Among these effects perturbations of the Ca^{2+} (Varečka and Carafoli 1982, Macara and Gray 1987, Richelmi et al 1989) and Na^+ homeostasis (Cantley et al 1982, Cassel et al 1984) have been reported Besides, some cell functions such as membrane potential changes (Zemková et al 1982, Lichtstein et al 1983) or stimulation of the secretion reaction (al-Laith and Pearce 1989, Lerea et al 1989, Churchill et al 1990, Zhang et al 1991) or of cell growth (Kanik-Ennulat and Neff 1990) have been found to be elicited by vanadate, these effects could be ascribed to perturbation of the Ca^{2+} homeostasis Among the mechanisms which have been shown to be affected by vanadate are ion pump activities (Barrabin et al 1989, Cruz-Mireles and Ortega-Blake 1990 Carmeli et al 1992), phosphorylation and/or dephosphorylation mechanism(s) (Earp et al 1983, Dong-Chang et al 1989, Lerea et al 1989, Křivánek and Novaková 1991, Andrews et al 1992, Pugazhenthi and Khandelwal 1992), phosphoinositide (Dong-Chang et al 1989 Catalan et al 1991, Bencheriff and Lukas 1992) and arachidonate (Levine 1991) metabolism, enzymes of the glucose metabolism (Cohen et al 1987, Percival et al 1990 Catalan et al 1991, Fillat et al 1992), cell redox reactions (Minasi and Willsky 1991 Byczkowski and Kulkarni 1992, Shi and Dalal 1992), DNA metabolism enzymes and DNA expression (Andrews et al 1992)

The results of our experiments designed to disclose the mechanism by which K^+ ions inhibit vanadate-induced $^{45}Ca^{2+}$ influx in human red blood cells (RBC) suggested that vanadate may modify properties of the Ca^{2+} influx pathway (Varečka et al 1997) The aim of the present work was to provide evidence, if any, for this possibility Evidence was obtained for the basal Ca^{2+} influx and the vanadate-induced Ca^{2+} uptake having different transport properties and suggesting that the effect of vanadate on the Ca^{2+} transport pathway involves changes in phosphoinositide lipid metabolism

Materials and Methods

Red blood cell suspension

Blood from healthy volunteers of both sexes was withdrawn by venipuncture into EDTA-containing anticoagulant (5 mmol/l final), and was used within 3 days, it was stored at 0–4 °C RBC were isolated after centrifugation of the blood (10 minutes at 600 × *g*) and aspiration of the supernatant with the buffy coat, three-fold washing

with and, finally, suspending into the medium containing (in mmol/l) 20 Tris-HCl, pH 7 3, 130 NaCl, 5 KCl, 10 glucose (further referred to as the suspension medium) to the final haematocrit of 30%, and immediately used for experiments

$^{45}Ca^{2+}$ influx induced by vanadate

The procedure described previously (Varečka and Carafoli 1982, Varečka et al 1986) was used Aliquots of 30% suspension were preincubated with 1 mmol/l $NaVO_3$ (further referred to as vanadate) for 15 min at 25 °C, followed by the addition of $^{45}Ca^{2+}$ (2 5 mmol/l final) and the incubation for 60 min at the same temperature The incubation was stopped by addition of the same volume of the stopping medium containing in mmol/l 20 Tris-HCl pH 7 3, 75 KCl, 60 NaCl, 10 glucose, and 1 EDTA, and by rapid centrifugation of the sample in a microcentrifuge The supernatant was sucked off and the pellet was washed with the same medium three more times Finally, the pellet was precipitated with 10% trichloroacetic acid (TCA) (w/v) containing 20 mmol/l $LaCl_3$, the precipitate was centrifuged and the pellet was taken for liquid scintillation counting Control cells without vanadate were treated in parallel All samples were run in duplicate and average values of parallel samples ± standard error are given in the Figures The standard error is indicated by bars when exceeding the dimension of the symbol Experiments shown in the Figures were typical of at least two (as a rule three) independent experiments

Measurement of the basal $^{45}Ca^{2+}$ influx

The procedure described by Pokudin and Orlov (1986) was modified as follows Red cells (30% haematocrit) were preincubated at 25 °C as described above, $^{45}Ca^{2+}$ (2 5 mmol/l, spec act approx 8 000 cpm/nmol) was added, and incubation was continued at 25 °C At times indicated (or after 20 min when not indicated specifically) 0 5 ml aliquots of the suspension were withdrawn and added to 5 ml of medium containing in mmol 2 5 EGTA, 70 KCl, 65 NaCl 10 glucose, 20 Tris-HCl, pH 7 3, cooled in an ice-water mixture, and immediately spun down on a microcentrifuge at 2–4 °C (the experiments were carried out in a cold room) Two more washing steps followed, and the radioactivity of the pellets was determined as described in the previous paragraph The substances to be tested were added 5 minutes prior to the addition of radionuclide

Analysis of phosphoinositides

Red cells (40% haematocrit) were incubated with $^{32}P_{in}$ (1 MBq/ml), for 60 min at 37 °C and membranes were prepared according to Dodge et al (1963) at 0–4 °C The membrane suspension was suspended in the suspension medium Aliquots of the suspension were supplemented with vanadate (1 mmol/l), or Ca^{2+} (2 5 mmol/l), or EGTA (2 5 mmol/l) as indicated in the autoradiogram, and were preincubated for

0, 10, and 60 min at 25 °C (the 10 min incubation is shown only) The incubation was stopped by the addition of Folch extraction reagent For the extraction and analysis of acidic phospholipids the procedure of Strunecká and Folk (1988) (HPTLC) was used except that the solvent system was chloroform methanol 20% methylamine = (60 35 10), according to Tysnes et al (1985) Additions are indicated at individual lanes (V stands for vanadate) Phospholipids derived from pig RBC are indicated by asterisks The identification of the spots was performed by R_f factors described by Tysnes et al (1985), by developing the same sample in different solvent systems

Chemicals

$^{45}CaCl_2$ was from Radiochemical Works (Swierk, Poland), ^{32}P-orthophosphate from Isocommerz (Dresden, Germany). Tris base, and dibutylphthalate from Serva (Heidelberg, Germany), valinomycin and *p*-chloromercuribenzoate (pCMB) from Sigma (St Louis, USA), and the methyl-phenyl silicone oil from Lučební závody Kolin (Czech Republic) $NaVO_3$ was a product of Reachim (Moscow, Russia) Nifedipine was synthesized at the Institute for Drug Research (Modra, Slovakia), and was kindly provided by Dr Zd Mahrla Tetrodotoxin, pertussis toxin and cholera toxin were purchased from Calbiochem (Luzern, Switzerland) Dimethylsulphoxide (DMSO) was obtained from Merck (Darmstadt, Germany), Tris(hydroxymethylamino) methane was purchased from Medika (Bratislava, Slovakia) Diclofenac – 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid, Diflunisal – [5-(2,4-difluorophenyl)salicylic acid], Etodolac – 1,8-diethyl 1,3,4,9-tetrahydropyrano(3,4-b) indole-1-acetic acid, Fenbufen – 4-(4-biphenylyl)-4-oxobutyric acid, Ibuprofen alpha-methyl-4-(2-methylpropyl)benzene acetic acid, Piroxicam – 4-hydroxy-2-methyl-N-2-pyridinyl-2H1,2-benzothiazine-3-carboxamide 1,1-dioxide, were all kindly provided by Dr J Grimová of the Institute of Pharmacy and Biochemistry (Prague, Czech Republic) Indomethacin – 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid was obtained from Léčiva (Prague, Czech Republic) Other chemicals used (all of analytical grade) were purchased from Lachema (Brno, Czech Republic)

Results

Properties of the Ca^{2+} transport without inducer

When RBC suspended in suspension medium were incubated with $^{45}Ca^{2+}$ under normal conditions (2 5 mmol/l final, 25 °C), and the extracellular radioactivity was washed out by repeated washings with EDTA-containing medium at room temperature, a labelling between 0 5–2 0 μmol/l_{cells} was obtained in individual experiments (Fig 1) The labelling remained constant during the experiments (usually 60 min) The addition of vanadate *prior* to the addition of $^{45}Ca^{2+}$ led to the increase of the $^{45}Ca^{2+}$ influx of the order(s) of magnitude (Fig 1) as shown before

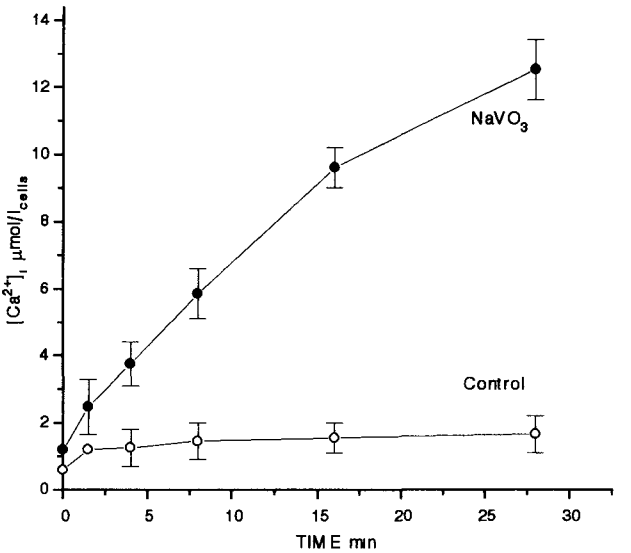

Figure 1. Kinetics of the vanadate-induced $^{45}Ca^{2+}$ transport and of the basal Ca^{2+} influx The procedures as described in Material and Methods were used for the measurement of vanadate-induced $^{45}Ca^{2+}$ influx without (open circles) and with (closed circles) pretreatment of RBC with 1 mmol/l vanadate

(Varečka and Carafoli 1982) When the more effective method for stopping the influx and pumping of $^{45}Ca^{2+}$ described by Pokudin and Orlov (1986) was used which consisted of a rapid cooling the suspension by diluting it in the 10-fold volume of EGTA-containing medium and rapid repeated washings at 0 °C, and using the radionuclide $^{45}Ca^{2+}$ with higher specific activity (about 8000 cpm/nmol), the kinetics of the labelling and factors which may influence it could be measured RBC were labelled by $^{45}Ca^{2+}$ in a very short time, reaching a steady-state level within about 3 minutes (Fig 2*A*), this suggested that the process of labelling reflects the transmembrane basal Ca^{2+} influx corresponding to the "resting" state of RBC Since the properties of the vanadate-induced $^{45}Ca^{2+}$ pathway have been described in detail (Varečka and Carafoli 1982, Varečka et al 1986, Varečka and Peterajová 1990) it was necessary to know whether or not the properties of the basal Ca^{2+} influx are identical to those induced by vanadate While the $^{45}Ca^{2+}$ influx induced by vanadate was strongly inhibited by increased extracellular K^+ concentrations (Fig 2*B*), the $^{45}Ca^{2+}$ influx observed in the absence of any inducer was not significantly changed when Na^+ in the suspension medium was isosmotically substituted with K^+ (Fig 2*A*) Choline$^+$ as a substitute stimulated the influx in

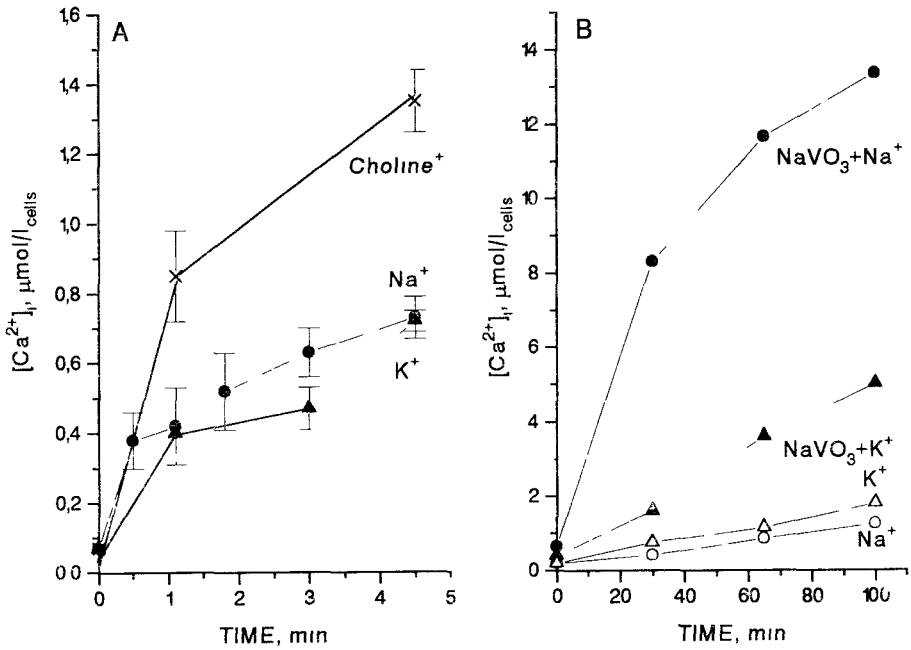

Figure 2 The response of basal $^{45}Ca^{2+}$ influx in human RBC (*A*) and of the vanadate induced $^{45}Ca^{2+}$ influx (*B*) upon the substitution of Na^+ by K^+ or $choline^+$ *A* Basal $^{45}Ca^{2+}$ influx in Na^+ rich (circles) K^+ rich (triangles) and $choline^+$-rich (crosses) media at 25°C *B* $^{45}Ca^{2+}$ influx in Na^+-rich (closed circles) and K^+-rich (closed triangles) media in the presence of vanadate Controls without vanadate Na^+ medium (open circles), K^+-medium (open triangles)

both vanadate-induced (see Fig 3 4 in Varečka et al 1997) and the basal $^{45}Ca^{2+}$ influx (Fig 2*A*) Valinomycin (1 μg/ml) slightly (by about 10%) stimulated the basal $^{45}Ca^{2+}$ influx in Na^+-containing medium both in the presence and in the absence of vanadate (not shown) Other inhibitors of Ca^{2+} influx differently affected both processes Nifedipin had only a weak inhibitory effect on the basal $^{45}Ca^{2+}$ influx but effectively inhibited the vanadate-induced influx (Fig 3*A*,*B*) Cu^{2+} ions and pCMB which were found to inhibit the vanadate induced $^{45}Ca^{2+}$ influx (Varečka et al 1986) were also ineffective in inhibiting the basal $^{45}Ca^{2+}$ influx (Fig 3 4,*B*) The basal $^{45}Ca^{2+}$ influx was inhibited by ruthenium red at concentrations above 1 μmol/l (not shown) In order to check whether the rate of the basal $^{45}Ca^{2+}$ influx reflects simple $^{45}Ca^{2+}$ diffusion across the RBC membrane into the cytoplasm or the activity of a putative Ca^{2+} carrier the saturatability of the $^{45}Ca^{2+}$ labelling with $^{45}Ca^{2+}$ was investigated, which is one of the criteria of carrier-mediated trans-

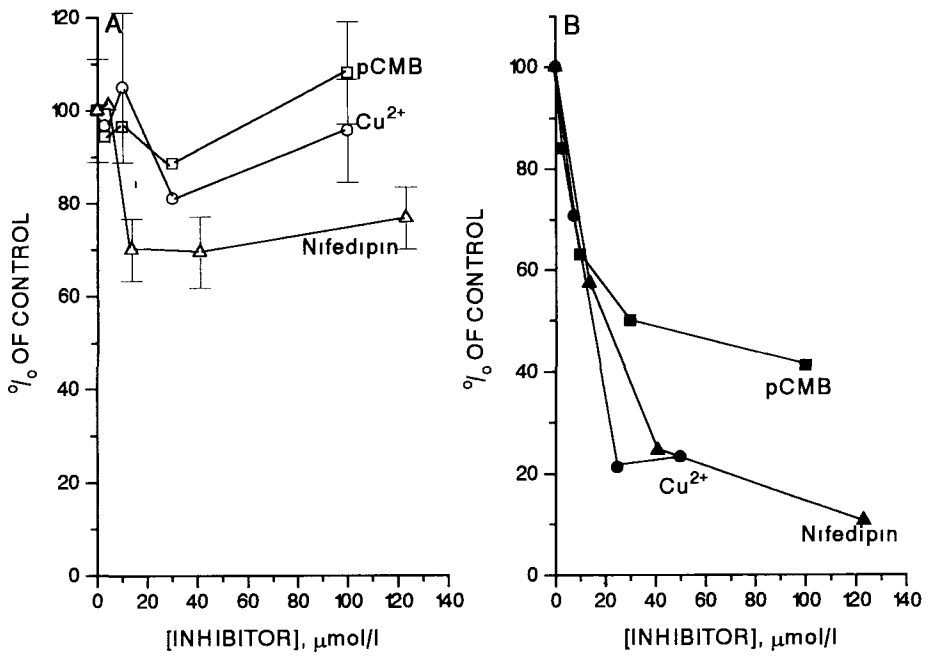

Figure 3. Effects of $^{45}Ca^{2+}$ transport inhibitors on the (*A*) basal $^{45}Ca^{2+}$ influx (*A*) and (*B*) $^{45}Ca^{2+}$ transport induced by vanadate in Na^+ media *A* Basal $^{45}Ca^{2+}$ influx in the presence of indicated concentrations of the following inhibitors Cu^{2+} (circles), pCMB (squares), nifedipin (triangles) 3 min incubation at 25 °C *B* $^{45}Ca^{2+}$ transport (1 mmol/l vanadate) in the presence of indicated concentrations of Cu^{2+} (circles), pCMB (squares) or nifedipin (triangles) 60 min incubation The same volume of vehicle (0 5% v/v) added to controls, water for Cu^{2+} and pCMB, DMSO for nifedipin Controls without vanadate were treated in parallel and the values were subtracted The effects of the inhibitors were tested in separate experiments

port The basal $^{45}Ca^{2+}$ influx was found to exhibit saturability, and its affinity for Ca^{2+} to be weaker than that of the vanadate-induced Ca^{2+} transport (Fig 4*A*, *B*) In experiments with the basal $^{45}Ca^{2+}$ influx a linear influx component appeared, the significance of which has not been investigated in detail as yet The linear component was also observed in some experiments with the vanadate-induced $^{45}Ca^{2+}$ influx mainly at higher vanadate concentrations than those used in the presented study (not shown) Similarly as the influx induced by vanadate (Varečka and Peterajová 1990) the basal $^{45}Ca^{2+}$ influx was inhibited by lower temperatures (not shown) When alternative methods such as repetitive washing without cooling, or chromatography of the cell suspension on a short column with CM-Sephadex, and rapid centrifugation were used, qualitatively the same results but lower values of

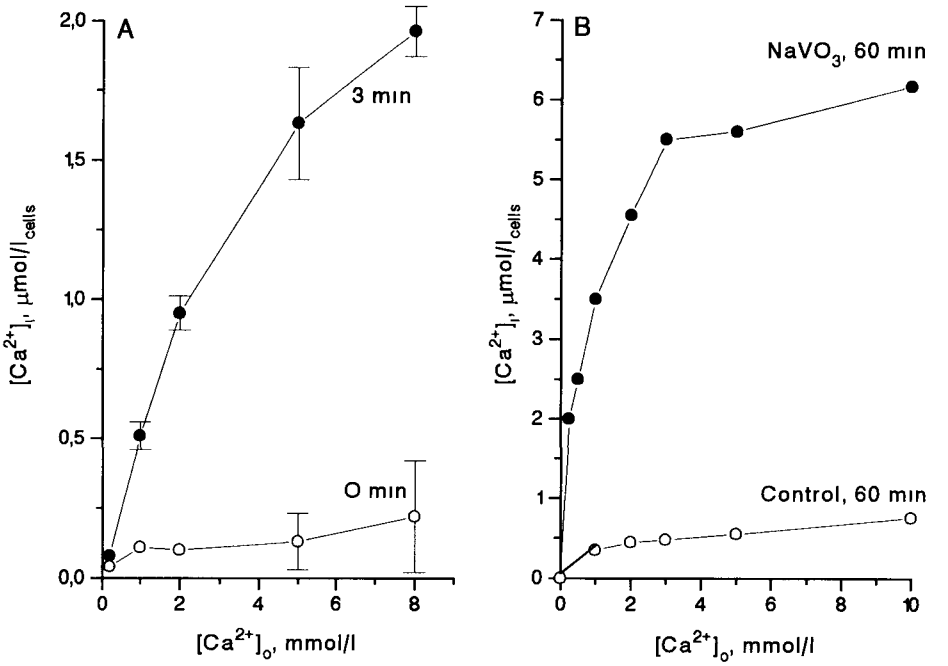

Figure 4. The dependence of the basal $^{45}Ca^{2+}$ influx (*A*) and of the vanadate-induced $^{45}Ca^{2+}$ influx (*B*) on the extracellular Ca^{2+} concentration *A* Basal $^{45}Ca^{2+}$ influx was measured for 3 minutes (closed circles) with concentrations of external $^{45}Ca^{2+}$ as indicated In control samples (open circles) the transport was measured for 0 minutes *B* $^{45}Ca^{2+}$ influx was measured for 60 minutes at 25 °C in the presence of 1 mmol/l vanadate (closed circles) In control samples (open circles) vanadate was omitted

Ca^{2+} influx were obtained suggesting that these methods did not sufficiently arrest the Ca^{2+}-pump activity

Possible mechanisms of changes in the Ca^{2+} influx pathway

In order to find out what mechanism is involved in the transformation of the Ca^{2+} influx pathway by vanadate several inhibitors of known signalling pathways were tested Pertussis toxin (up to 5 µg/ml, cholera toxin (up to 10 µg/ml) (blockers of the G-protein function) did not influence the vanadate-induced Ca^{2+} influx (Table 1) The Gárdos effect was not affected by these agents either (not shown) Inhibitors of arachidonate oxygenation (non-steroidal antiinflammatory agents) had inhibitory effects on the vanadate-induced Ca^{2+} influx in the $10^{-4} - 10^{-3}$ mol/l range but some members of this class of drugs displayed opposite effects (acetylsalicylic acid and etodolac (Table 1) The inhibitor of the Na^+-linked action poten-

Table 1. Effects of inhibitors of the signalling pathways on the vanadate-induced $^{45}Ca^{2+}$ uptake

Inhibitor	Concentration	Effect
Pertussis toxin ($n = 1$)	max 5 μg/ml	No effect
Cholera toxin ($n = 2$)	max 10 μg/ml	No effect
Tetrodotoxin ($n = 2$)	max 40 μg/ml	No effect
Acetylsalicylic acid ($n = 2$)	max 1 mmol/l	Stimulation 15%
Ibuprofen ($n = 3$)	max 0 1 mmol/l	Inhibition 27%
Indomethacin ($n = 2$)	max 1 mmol/l	Inhibition $IC_{50} = 140$ μmol/l
Diclofenac ($n = 2$)	max 0 1 mmol/l	Inhibition $IC_{50} = 100$ μmol/l
Piroxicam ($n = 6$)	max 0 1 mmol/l	Inhibition 25 40%
Diflunisal ($n = 2$)	max 0 1 mmol/l	Inhibition 40%
Fenbufen ($n = 2$)	max 0 1 mmol/l	Inhibition $IC_{50} = 90$ μmol/l
Etodolac ($n = 3$)	max 0 1 mmol/l	Stimulation 28%
LiCl* ($n = 5$)	15 mmol/l	Inhibition 45%

The non-steroidal anti-inflammatory drugs were dissolved in dimethylsulphoxide and were added into the suspension under vigorous stirring The final concentration of DMSO was the same in samples including controls – 0 5% (v/v) *LiCl was added to the whole blood and incubated for 72 h at 4 °C RBC were isolated and the $^{45}Ca^{2+}$ influx was measured in the presence of the same concentration of LiCl Control RBC without LiCl were treated in parallel

tial, tetrodotoxin, had no effect up to 40 μg/ml Li^+ ions inhibited the $^{45}Ca^{2+}$ influx when preincubated with whole blood for several days, and the same concentration of Li^+ was kept during the isolation and the transport measurements

Our earlier observation (Varečka et al 1986) showed that there is no vanadate-induced $^{45}Ca^{2+}$ influx in pig RBC As these cells are known to be defective in the phosphoinositide (PI) metabolism (Allan and Michell 1977) the possibility was tested that changes in PI metabolism may underly the altered Ca^{2+} influx pathway properties The analysis of the $^{32}P_{in}$ radioactivity incorporation after separating phospholipids according to Tysnes et al (1985) into PI derivatives and phospha-

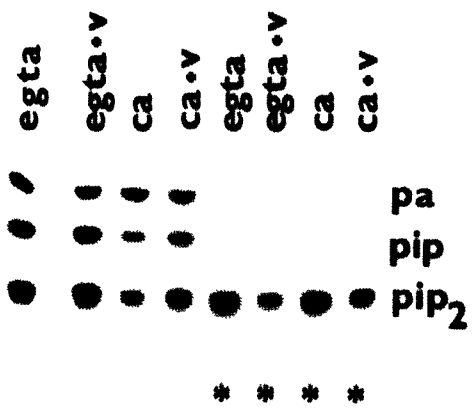

Figure 5. The ^{32}P orthophosphate incorporation into polyphosphoinositides in human and pig RBC, and its modification by vanadate and Ca^{2+} withdraval Membranes from human and pig (asterisks) RBC labelled with $^{32}P_{in}$ were prepared as described in Materials and Methods, and aliquots were supplemented with vanadate (V) (1 mmol/l) or $^{40}Ca^{2+}$ (2 5 mmol/l) or EGTA (2 5 mmol/l) After 10 min preincubation at 25 °C the reaction was stopped by Folch reagent and phospholipids were isolated and analysed as described in Materials and Methods

tidic acid (PA) showed that in human RBC vanadate stimulated the incorporation into PIP_2 and PIP both in the presence of EGTA and Ca^{2+} (Fig 5) Under identical conditions pig RBC showed the vanadate-induced decrease in the radioactivity incorporation into PIP_2 but only minute or no radioactivity was found in PIP and PA, respectively (Fig 5) In both cell types EGTA alone stimulated the radioactivity incorporation into PIP_2 (and PIP in human RBC) but not into PA as compared with the Ca^{2+} treatment

Discussion

The results presented show that the properties of the Ca^{2+} influx in human RBC observed in the absence and in the presence of vanadate differ from each other in their transport characteristics In the absence of vanadate RBC were labelled with $^{45}Ca^{2+}$ to the extent approaching 1 $\mu mol/l_{cells}$-an observed level of the RBC Ca^{2+} (Varečka and Carafoli 1982) (Fig 1*A*) The extent of labelling corresponds to the "resting" cytoplasmic Ca^{2+} content which is higher than the cytoplasmic Ca^{2+} concentration (Yoshida et al 1986) The difference may reflect the fact that a part of the Ca^{2+} content is represented by a bound pool (Yoshida et al 1986, Engelmann

and Duhm 1987) The slow rise of the Ca^{2+} content observed in our conditions (a linear component) (Fig 2*B*) is probably a consequence of the measurement at 25 °C which necessarily led to a decrease of the Ca^{2+}-ATPase activity

The results also show that the mechanism by which vanadate influences the Ca^{2+} homeostasis also include the direct modification of properties of the Ca^{2+} transport which conveys Ca^{2+} into the RBC interior Results presented here confirm the suggestion by Fuhrmann et al (1984) of multiple target sites of vanadate in the exhaustive explanation of the vanadate-induced $^{45}Ca^{2+}$ influx Our data indicate that also in the absence of any perturbing agent the basal Ca^{2+} influx is mediated by a carrier mechanism (which together with the Ca^{2+}-ATPase create the basal cyclic flow of Ca^{2+} ions) but its precise characterization still remains open Also, we have been unable as yet to decide conclusively whether the immediate cause of the vanadate effect is a direct modification of the molecule of the carrier or activation of a different carrier with different properties The second possibility could be favoured taking into the account the results of Engelmann and Duhm (1989) Another possibility which emerges from our previously published experiments is that the increased sensitivity to dihydropyridines and to HS-reagents is linked to the process of the activation of the Ca^{2+}-activated K^+ channel (K(Ca)) by vanadate since the Ca^{2+}-binding step is preceded by a temperature-dependent process (Varečka and Peterajová 1990) and also since K(Ca) represents a Ca^{2+} influx supporting element in the vanadate – treated RBC (Varečka et al 1997) The data presented herein cannot discriminate between these possibilities and, probably, only isolation and reconstitution of the putative carriers could be helpful with this respect

The chemical mechanisms underlying the changes in the RBC passive Ca^{2+} pathway induced by vanadate may involve one or more signalling pathways The role of G-proteins in the vanadate action seems to be excluded This could be inferred from the data in Table 1 It should be mentioned that the strong inhibitory effects of PTX and TTX in corresponding concentrations on the fluoride-induced and Ca^{2+}-dependent increase of Na^+ membrane permeability (Varečka et al 1995) suggest that the choice and concentrations of toxins used were correct, and that their targets are present in human RBC but do not participate in the action of vanadate The role of oxygenated arachidonate metabolites is not unequivocally supported by the action of non-steroidal anti-inflammatory agents which act at relatively high concentrations and, moreover, their action is dual (acetylsalicylic acid and etodolac vs other drugs) (Table 1) The involvement of arachidonate metabolites in the action of vanadate has to be investigated by independent approaches The most probable mechanism involved in the action of vanadate is PI metabolism as suggested by the effects of EGTA and vanadate on the phosphate turnover in polyphosphoinositides (or on their content), by the differences between pig and human RBC (Fig 5), and by the effect of Li^+ (Table 1) Similar effects

of vanadate were observed earlier by Memon and Boss (1989) in plant tissue, and they may reflect a common mechanism

In our study of the vanadate-induced Ca^{2+} influx interesting interspecies-differences of the RBC response to vanadate were observed Whereas pig RBC did not exhibit vanadate-induced $^{45}Ca^{2+}$ influx (Varečka et al 1986) and are defective in the phosphoinositide metabolism (Allan and Michell 1977) (Fig 5), dog RBC displayed a massive $^{45}Ca^{2+}$ influx when compared with human RBC (Varečka et al 1997) Thus, analysis of interspecies differences of PI metabolism could be the most promising tool for the understanding of the vanadate-induced ion permeability changes

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