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EFFECTS OF VASODILATOR DRUGS, ALKALINE PHOSPHATASE, AND CYCLIC AMP-DEPENDENT PROTEIN KINASE ON THE ^{45}CALCIUM UPTAKE OF SARCOLEMMAL MICROSOMES FROM HUMAN UMBILICAL ARTERIES

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1 A microsomal fraction was prepared from human umbilical arteries by differential centrifugation. The preparation was capable of an oxalate-stimulated Ca^{2+} uptake at a mean rate of 0.74 nmol Ca^{2+} mg^{-1} protein min^{-1} which could be inhibited by a Ca^{2+} ionophore, A 23 187, and by Tween 80.

2 The rate of Ca^{2+} uptake in the fractions obtained by density gradient fractionation paralleled 5'-nucleotidase activity suggesting that vesicles of predominantly sarcolemmal origin were responsible for the microsomal Ca^{2+} uptake.

3 Cyclic adenosine 3',5'-monophosphate-dependent protein kinase enhanced membrane phosphorylation but did not affect Ca^{2+} uptake. Preincubation with alkaline phosphatase reduced membrane phosphorylation to a greater extent than Ca^{2+} uptake. These data are not in favour of a close correlation between Ca^{2+} uptake and phosphorylation.

4 None of 15 vasodilator drugs (bencyclane, carbocromen, diazoxide, dilazep, hydralazine, indapamide, isosorbide dinitrate, methyl-isobutyl-xanthine, minoxidil, naftidrofuryl, nitroglycerine, prenylamine, sodium nitroprusside, tetracaine, and verapamil) had any effect on Ca^{2+} uptake at 10^{-5} M. This suggests that vasodilator drugs do not act by a direct influence on the Ca^{2+} pumps of vascular smooth muscle cells.

Introduction

In the vascular smooth muscle cell, relaxation results from a reduction of the cytoplasmic free calcium concentration either by Ca^{2+} uptake into intracellular storage sites, or by Ca^{2+} extrusion across the cell membrane. For the latter, a Na^{+}–Ca^{2+}-exchange mechanism had been postulated (Reuter, Blaustein & Haeusler, 1973), but more recent data suggest the existence of an adenosine triphosphate (ATP)-dependent Ca^{2+} outward pump at the level of the cell membrane (van Breemen, 1976; Wei, Janis & Daniel, 1976).

We have prepared a microsomal fraction from human umbilical arteries. Sucrose density gradient fractionation, and assays of marker enzymes revealed its predominantly sarcolemmal origin. The effects of alkaline phosphatase, and of cyclic adenosine 3',5'-monophosphate (cyclic AMP)-dependent protein kinase (cyclic AMP-PK) on its Ca^{2+} uptake, and phosphorylation were studied. Since the mechanism of action of vasodilator drugs may depend on a modulation of the activity of the sarcolemmal Ca^{2+} pump, we have, in addition, investigated the influence of 15 vasodilator drugs on microsomal Ca^{2+} uptake.

Some of these results have been presented at the IIIrd International Congress on Vascular Neuroeffector Mechanisms (Louvain/Belgium, July, 1978; Kreye & Schlicker, 1980), and at the 20th Spring Meeting of the German Pharmacological Society (Kreye & Schlicker, 1979).

Methods

Preparation of microsomes, and density gradient fractionation

Human umbilical arteries were homogenized in a medium containing 2.5 mM phosphate buffer or 5 mM Tris-maleate buffer pH 7.0, 100 mM KCl, and 2 mM disodium edetate (EDTA) with an Ultra-Turrax for 15 s, and then with a Potter-Elvehjem homogenizer for 2.5 min. The homogenate was centrifuged at 4500 *g* for 15 min, the supernatant for 15 min at 11,000 *g*, and again the supernatant at 50,000 *g* for 80 min. For density gradient fractionation, the homogenate was centrifuged at 4500 *g* for 15 min, and the supernatant at 100,000 *g* for 30 min. The resulting pellet was suspended in homogenization medium, and layered on a discontinuous sucrose density gradient consisting of 38, 32, 29 and 26% layers. After centrifugation at

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110,000 *g* for 2 h, 4 fractions were withdrawn, diluted, and sedimented at 100,000 *g* for 30 min. The pellets were suspended in 100 mM KCl. All procedures were carried out at 4°C. Protein was determined according to Lowry, Rosebrough, Farr & Randall (1951).

Ca^{2+} uptake, and phosphorylation experiments

Usually, Ca^{2+} uptake was studied at 37°C in an incubation medium containing 20 mM histidine buffer pH 6.6, 100 mM KCl, 5 mM $MgCl_2$, 5 mM K^+ oxalate, 5 mM ATP, 5 μM ruthenium red, 0.1 mM $^{45}CaCl_2$, and 0.2 mg microsomal protein/ml. At the times scheduled, aliquots were subjected to the filtration technique described elsewhere (Kreye, Baron, Lüth & Schmidt-Gayk, 1975), and their radioactivity determined by liquid scintillation counting. When alkaline phosphatase was studied, microsomes were preincubated for 20 min with the enzyme, and $CaCl_2$ and ATP added subsequently to start the Ca^{2+} uptake.

Phosphorylation was assayed at 37°C in a similar medium, but containing 50 μM [γ-^{32}P]-ATP, an ATP-regenerating system (0.2 mM PEP and 20 μg/ml pyruvate kinase), and unlabelled $CaCl_2$. After 20 min, aliquots were pipetted on Whatman GF/C filters which were immersed twice in a stirred solution of 10% TCA, 1% Na^+ pyrophosphate and 1% KH_2PO_4 for 15 min, followed by counting of their radioactivity. To rule out phosphorylation of actomyosin possibly present in the microsomal fraction, a control experiment was carried out with a preparation submitted to an additional purification step as described by Nonomura & Ebashi (1975).

Enzyme assays

The various marker enzymes (see Results) were determined by using standard methods. The effect of 10^{-7} to 10^{-3} M Ca^{2+} (10^{-7} to 10^{-5} M adjusted with EGTA) on ATPase activity was measured at room temperature by the coupled enzyme test based on pyruvate kinase, PEP, lactic dehydrogenase, and NADH. In another study, ATPase was measured at 37°C by determining P_i liberated (Rockstein & Herron, 1951) in the medium used for Ca^{2+} uptake studies, but containing either 10^{-4} M unlabelled $CaCl_2$ or no $CaCl_2$ (plus 0.15 mM EGTA).

Materials

The drugs used were A 23 187 (Eli Lilly), bencyclane (Thiemann), carbocromen (Cassella-Riedel), diazoxide (Byk-Essex), dilazep (Asta), hydralazine (Ciba), indapamide (Servier), isosorbide dinitrate (Pharma Schwarz), mersalyl, prenylamine, tetracaine (all Hoechst), 1-methyl-3-isobutyl-xanthine (EGA-Chemie), minoxidil (Upjohn), naftidrofuryl (Lipha), nitroglycerine, sodium nitroprusside (both Merck), Tween 80 (Atlas), and verapamil (Knoll). The enzymes used were pyruvate kinase, and lactic dehydrogenase (both Boehringer Mannheim), porcine and bovine alkaline phosphatase (Sigma or Serva). Cyclic AMP-PK (sp. act.: 95 nmol phosphate min^{-1} mg^{-1} wet wt., histone II A as substrate), and pure cyclic AMP-PK II catalytic subunit from bovine heart muscle were kindly provided by Dr F. Hofmann, Department of Pharmacology, University of Heidelberg. $^{45}CaCl_2$ was purchased from Amersham Buchler, and [γ-^{32}P]-ATP from NEN.

Statistical methods

Results are expressed as means ± s.e. For statistical evaluations Student's *t* test or the Wilcoxon test, and for the comparison of more than two samples the analysis of variance or the Friedman two-way analysis by ranks were applied.

Results

Characterization of the microsomal fraction

By *differential centrifugation*, a yield of 1.25 ± 0.03 mg microsomal protein was obtained from 1 g wet wt. of arterial tissue ($n = 111$). The preparation consisted of smooth membranes forming closed vesicles of variable size (Figure 1). In comparison to the crude homogenate or the 11,000 *g* pellet, the plasma membrane marker, 5'-nucleotidase, was found enriched, and the mitochondrial marker, cytochrome c oxidase, reduced, respectively (Table 1).

The determinations based on *density gradient centrifugation* revealed that the degree of Ca^{2+} uptake paralleled the activity of the sarcolemmal marker, 5'-nucleotidase, among the fractions; both had their peak in F_2 (Figure 2).

Characterization of microsomal Ca^{2+} uptake

The Ca^{2+} uptake varied considerably in the single experiments at a mean rate of 0.74 ± 0.05 nmol min^{-1} mg^{-1} microsomal protein ($n = 74$). It was linearly related to the concentration of microsomal protein in the incubation medium ($r = 0.996$), and was highly dependent on the presence of ATP, temperature and pH (Figure 3). Oxalate, as a precipitant, strongly augmented the microsomal Ca^{2+} uptake, and this effect was inhibited by a Ca^{2+} ionophore, A 23 187 (Figure 3). The detergent Tween 80 (0.06% v/v), reduced Ca^{2+} uptake from 45.9 ± 4.7 to 9.1 ± 1.8

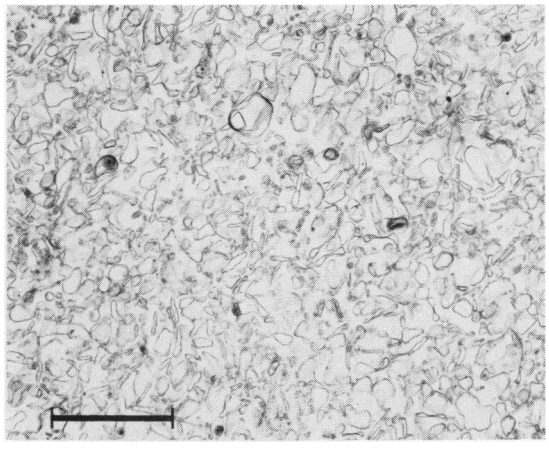

Figure 1 Electron micrograph of a microsomal fraction isolated from human umbilical arteries. Following preparation and homogenization, three centrifugation steps were performed (4500 *g*/15 min; 11,000 *g*/15 min; 50,000 *g*/80 min). The 50,000 *g* pellet was stirred in ice-cold 0.1 M KCl for 30 min, and then spun at 56,000 *g* for 60 min. The final pellet was immersed in Karnovsky fixative, and micrographed by Prof. Dr W. G. Forssmann, IIIrd Anatomical Institute, University of Heidelberg. The bar represents 1 μm.

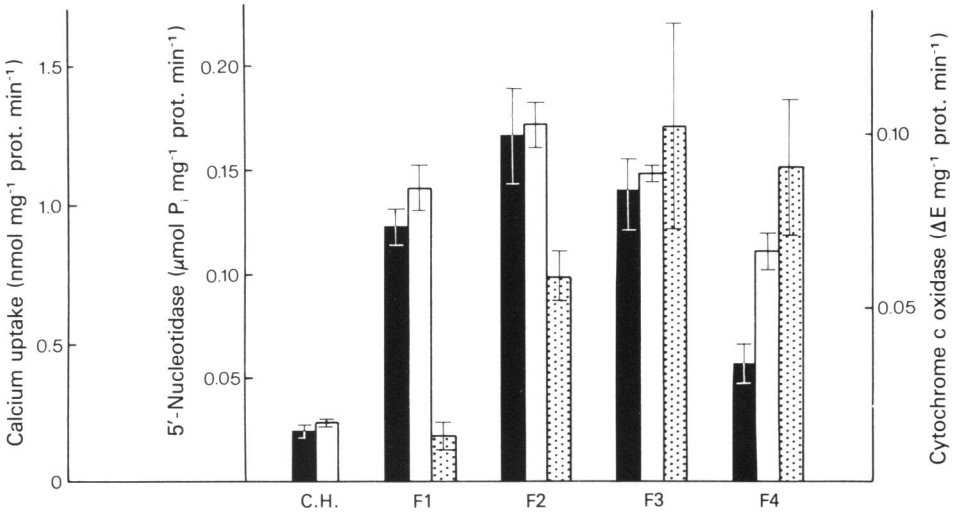

Figure 2 Specific activities of Ca^{2+} uptake (solid columns), 5'-nucleotidase (open columns), and cytochrome c oxidase (stippled columns) in the crude homogenate (C.H.) of human umbilical arteries, and in 4 subfractions obtained by discontinuous density gradient centrifugation. Subfractions: F 1 = top layer and interfacial zone to 26% sucrose; F 2 = 26% layer and interfacial zone to 29% sucrose; F 3 = 29% layer and interfacial zone to 32% sucrose; F 4 = 32% layer and interfacial zone to 38% sucrose. Mean values are shown ($n = 3 - 4$); vertical lines show s.e. mean. Note that cytochrome c oxidase activity could not be measured in the crude homogenate because of the presence of large particles.

nmol Ca^{2+} mg^{-1} protein 30 min^{-1} ($n = 4$), i.e. by 80%. The mercury compound, mersalyl, similarly inhibited the Ca^{2+} uptake at an ID_{50} of 4.5×10^{-6} M. No significant effect of 10^{-7} to 10^{-3} M Ca^{2+} on the microsomal ATPase activity was observed (Table 1), whereas in control experiments with microsomes from rabbit skeletal muscle, Ca^{2+} induced a striking increase in ATPase activity.

Table 1 Enzymatic activities of microsomal and other fractions from human umbilical arteries obtained by differential centrifugation

Enzymes	*Temperature* (°C)	n	*Microsomes*	*Other fractions*
Sarcolemmal markers				*Crude homogenate*
5'-Nucleotidase[a]	37	4	0.52 ± 0.13	0.15 ± 0.07
Ouabain-sens. K^+-phosphatase[b]	37	4	57.2 ± 3.0	22.7 ± 7.0
Alkaline phosphatase[b]	37	4	10.5 ± 1.9	3.7 ± 1.1
Sarcoplasmic reticulum marker				
Glucose-6-phosphatase[a]	37	6	Not found	
Mitochondrial marker				*11,000 g pellet*
Cytochrome c oxidase[c]	22	3	0.5 ± 0.3	1.9 ± 0.1
ATPase[a,d], no Ca^{2+}	22	7	2.07 ± 0.30	
10^{-7} M Ca^{2+}	22	7	2.05 ± 0.24	
10^{-6} M Ca^{2+}	22	7	2.01 ± 0.24	
10^{-5} M Ca^{2+}	22	7	1.94 ± 0.21	
10^{-4} M Ca^{2+}	22	7	2.21 ± 0.26	
10^{-3} M Ca^{2+}	22	7	1.65 ± 0.21	
ATPase[a,e], no Ca^{2+}	37	7	4.80 ± 0.89	
10^{-4} M Ca^{2+}	37	7	4.44 ± 0.94	

a = μmol P_i mg^{-1} protein 30 min^{-1}; b = nmol *p*-nitrophenol mg^{-1} protein 30 min^{-1}; c = ΔE mg^{-1} protein 30 min^{-1}; d = no significant differences, analysis of variance: $\hat{F} = 0.58 < F_{5;36;0.05} = 2.47$; e = no significant difference, Student's *t* test: $\hat{t} = 0.28 < t_{0.05;12} = 2.18$.

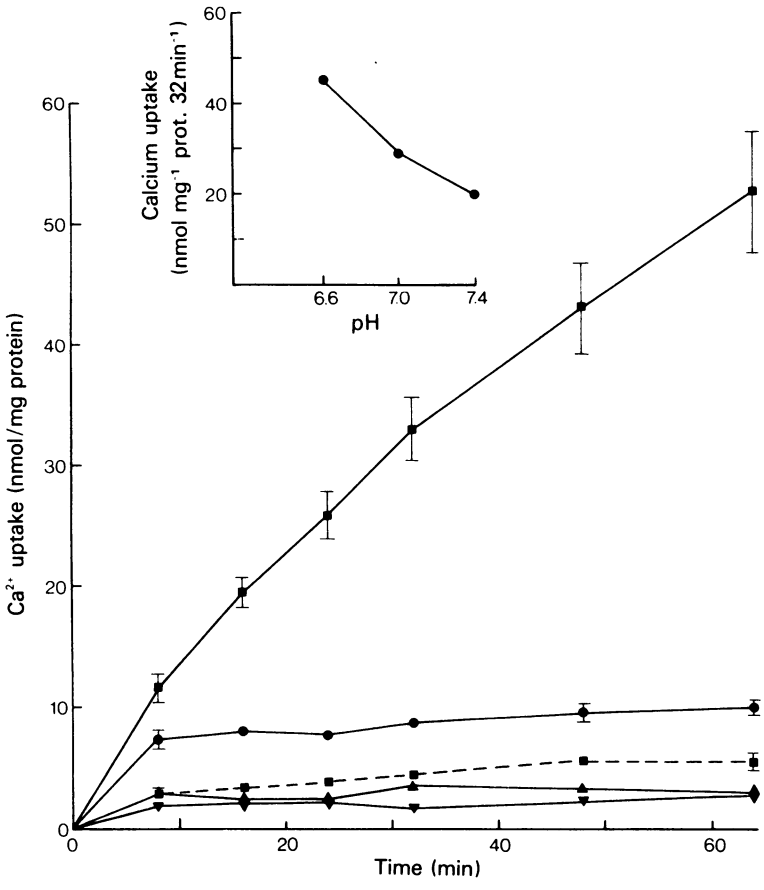

Figure 3 Effects of ATP, oxalate, A 23 187, temperature, and pH (inset) on Ca^{2+} uptake by a microsomal fraction isolated from human umbilical arteries. Experimental conditions: 37°C, ATP, and oxalate omitted (▼); 3°C, ATP, and oxalate present (▲); 37°C, ATP, oxalate, and 10^{-5} M A 23 187 present (■, broken line); 37°C, ATP present, oxalate omitted (●); 37°C, ATP, and oxalate present (■, solid line). Values are means of $n = 3$–7, vertical lines show s.e. mean; in the lower curves, s.e. values are mostly contained within the diameter of the symbols. Inset values are means of $n = 2$.

Effect of alkaline phosphatase, and of cyclic AMP-PK on Ca^{2+} uptake and phosphorylation

Preincubation with bovine alkaline phosphatase resulted in a concentration-dependent and significant reduction of Ca^{2+} uptake by up to 27% of the control value (Figure 4). In another experiment in which 0.02, 0.04, 0.06 and 0.2 u/ml of porcine alkaline phosphatase were used, reductions of Ca^{2+} uptake (control: 44.05 nmol mg^{-1} protein 30 min^{-1}) by 12.8, 15.3, 19.9 and 40.1%, respectively, were found. ATP splitting by 1 u/ml of bovine alkaline phosphatase in the absence of microsomes did not exceed 5% in 20 min. Thus, consumption of ATP by this enzyme does not underlie its inhibitory effect on the Ca^{2+} uptake. The phosphorylation of microsomal protein was diminished in the presence of bovine alkaline phosphatase (Table 2).

Cyclic AMP plus cyclic AMP-PK as well as the cyclic AMP-PK catalytic subunit in various concentrations had no effect on Ca^{2+} uptake of untreated or alkaline phosphatase-pretreated microsomes (Table 2). The same result was obtained when the ATP concentration was reduced from 5 mM to 0.5 or 0.05 mM in the incubation medium. In contrast, the phosphorylation of microsomal protein was considerably enhanced by cyclic AMP-PK or its catalytic subunit in the standard preparation as well as in a microsomal fraction cleared from possible contamination with arterial actomyosin (Table 2).

Effects of vasodilator drugs on Ca^{2+} uptake

Diazoxide, hydralazine, isosorbide dinitrate, methyl-isobutyl-xanthine, nitroglycerine, prenylamine, and

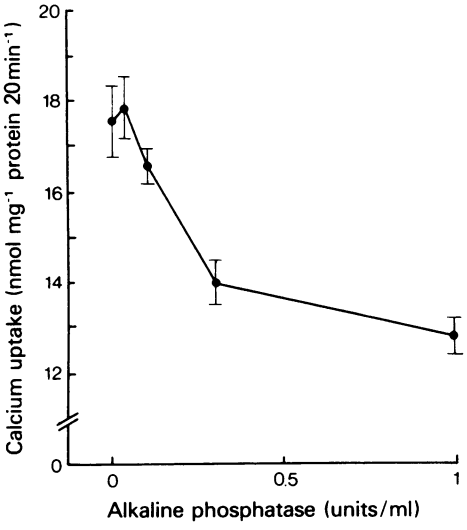

Figure 4 Effect of pre-incubation for 20 min with various concentrations of bovine alkaline phosphatase on Ca^{2+} uptake by a microsomal fraction isolated from human umbilical arteries. Applying the Friedman test, the differences proved to be statistically significant ($\hat{\chi}^2_R = 16.48 > \chi^2_{R(4;\,0.005)} = 14.86$). Values are means of $n = 5$; vertical lines show s.e. mean.

sodium nitroprusside, all at 10^{-5} M, did not influence the time course of Ca^{2+} uptake irrespective of whether the incubations were carried out in the standard medium, or in the absence of oxalate, or without both oxalate and ATP (Figure 5a and b). In another series of experiments, 10^{-5} M of these drugs as well as of bencyclane, carbocromen, dilazep, nafidrofuryl, tetracaine, verapamil (all water-soluble), indapamide, and minoxidil (both ethanol-soluble) had no statistically significant effect on the Ca^{2+} uptake of the microsomes incubated in standard medium for 30 min ($n = 4$–5).

Discussion

The electron micrograph of the microsomal fraction of human umbilical arteries as well as the activation by a precipitant, oxalate, and the inhibition by a detergent, Tween 80, and a calcium ionophore, A 23 187, of its Ca^{2+} uptake, demonstrate that it consists of sealed vesicles. The origin of microsomes isolated from vascular smooth muscle is difficult to determine since a reliable marker for its intracellular membranes is lacking (Thorens & Haeusler, 1978; Wuytack, Landon, Fleischer & Hardman, 1978). The characterization of the microsomal fraction by density gradient centrifugation suggests that vesicles predominantly of sarcolemmal origin are responsible for its Ca^{2+} uptake.

A close correlation between Ca^{2+} uptake and phosphorylation in microsomes from rat uterus has been described (Nishikori, Takenaka & Maeno, 1977). As in the experiments of Clyman, Manganiello, Lovell-Smith & Vaughan (1976), however, microsomes from human umbilical arteries were not stimulated by cyclic AMP-PK, even when we used a pure preparation of its catalytic subunit. In contrast, the incorporation of ^{32}P was strongly augmented by cyclic AMP-PK. Thus, its failure to stimulate Ca^{2+} uptake cannot be explained by an already complete phosphorylation of the microsomes. Regarding this lack of correlation

Table 2 Effects of cyclic AMP plus cyclic AMP-PK or its catalytic subunit and alkaline phosphatase (AP) on the Ca^{2+} uptake and phosphorylation of microsomes isolated from human umbilical arteries

AP (*u*/ml)	*Cyclic AMP-PK* (μg/ml)	*Ca^{2+} uptake* (nmol Ca^{2+} mg^{-1} prot. min^{-1})			*Phosphorylation* (pmol P mg^{-1} prot. min^{-1})		
—	—	1.21 ± 0.13[a]	1.12	0.76	2.36	2.94[b]	2.46[c]
—	30 (+10^{-6} M cyclic AMP)	1.27 ± 0.13[a]					
—	300 (+5 × 10^{-6} M cyclic AMP)			0.73	2.72		
—	1.35 (cat. subunit)					4.73[b]	5.45[c]
—	2.70 (cat. subunit)					5.10[b]	
—	5.40 (cat. subunit)		1.18			7.48[b]	
1.0	—			0.55	0.79		1.69[c]
1.0	300 (+5 × 10^{-6} M cyclic AMP)			0.53	1.30		
1.0	1.35 (cat. subunit)						3.61[c]

[a] $\bar{x}$ ± s.e., $n = 6$, not significantly different; [b]correlation coefficient, $r = 0.984$; [c]phosphorylation measured in a purified microsomal preparation (see text).

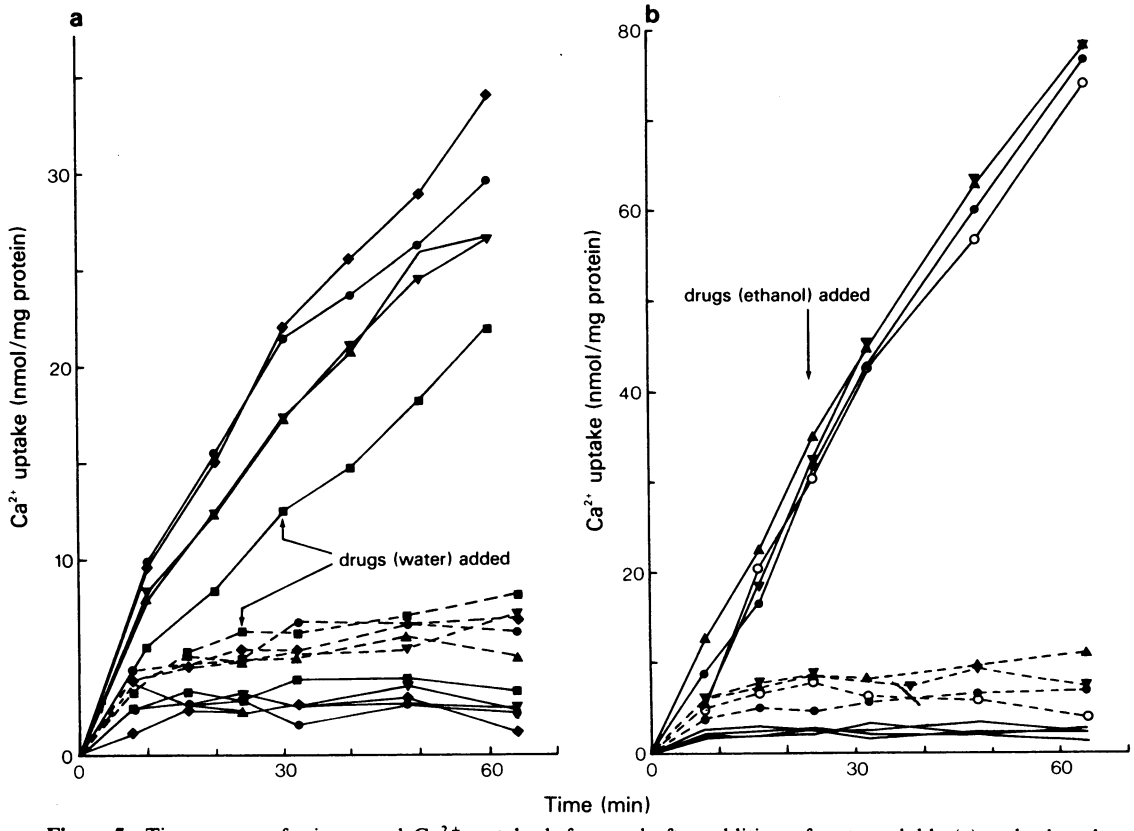

Figure 5 Time course of microsomal Ca^{2+} uptake before and after addition of water-soluble (a) and ethanol-soluble (b) vasodilator drugs at a concentration of 10^{-5} M. The three sets of curves in either figure correspond to the following experimental conditions: upper curves (solid lines)—standard conditions, i.e. 37°C, ATP, and oxalate present; intermediate curves (broken lines)—oxalate omitted; lower curves (solid lines)—ATP, and oxalate omitted. Water-soluble drugs or water (control; a) as well as ethanol-soluble drugs or ethanol (control; b) were added immediately after withdrawal of the third aliquot to the incubate (time points indicated by arrows). In (a): (●) Control; (◆) hydralazine; (▲) methyl-isobutyl-xanthine; (■) prenylamine; (▼) sodium nitroprusside. In (b): (○) control; (▼) diazoxide; (●) isosorbide dinitrate; (▲) nitroglycerine.

between cyclic AMP-PK-induced phosphorylation, and Ca^{2+} uptake (which has also been described for canine aortic microsomes; Allen, 1977), our finding of a reduced Ca^{2+} uptake in the presence of alkaline phosphatase does not necessarily reflect an effect of the reduced phosphorylation with this enzyme. However, it is of interest in so far as a reciprocal relationship between plasma membrane alkaline phosphatase activities, and ATP-dependent Ca^{2+} uptake capacities in vascular microsomes from rats with various forms of hypertension has been found recently (Kwan, Belbeck & Daniel, 1979).

In a previous study in our laboratory, some vasodilators (dilazep, naftidrofuryl, sodium nitroprusside, verapamil) in concentrations of 10^{-6} to 10^{-5} M had been found to inhibit slightly the Ca^{2+} uptake of a poorly defined microsomal fraction from rabbit aorta (Baron & Kreye, 1973; Kreye *et al.*, 1975). In our present study on microsomes from human umbilical arteries and of predominantly sarcolemmal origin, none of 15 vasodilator drugs and vasoactive antihypertensives, including the 4 drugs used in the former study, showed any effect on the microsomal Ca^{2+} uptake at 10^{-5} M. Negative results with some of these drugs at the same concentration were also obtained in studies on microsomes from bovine carotid arteries (Klinner, Ehlers, Fermum & Meisel, 1977), or from rabbit aorta (Thorens & Haeusler, 1979). Only at considerably higher concentrations (10^{-4} M or more) did a few of these drugs inhibit the microsomal Ca^{2+} uptake slightly, but this seems to be of little relevance for the understanding of the mode of action of these drugs. Moreover, a stimulation rather than an inhibition of cellular calcium pumps would be in keeping

with a drug-induced reduction of the cytoplasmic calcium concentration, thereby causing relaxation of vascular smooth muscle. The lack of such an effect of vasodilator drugs suggests that they do not directly affect the activity of cellular Ca^{2+} pumps, or that they act upon components of the contraction-relaxation cycle other than active Ca^{2+} sequestration or extrusion mechanisms.

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References

ALLEN, J.C. (1977). Ca^{2+}-binding properties of canine aortic microsomes: lack of effect of cAMP. *Blood Vessels*, **14**, 91–104.

BARON, G.D. & KREYE, V.A.W. (1973). Effects of drugs on $^{45}Ca^{2+}$ uptake by isolated vascular smooth muscle membranes. *Pflügers Arch.*, Suppl. to **343**, R 54.

BREEMEN, C. VAN (1976). Transmembrane calcium transport in vascular smooth muscle. In *Vascular Neuroeffector Mechanisms*. ed. Bevan, J.A., Burnstock, G., Johansson, B., Maxwell, R.A. & Nedergaard, O.A. pp. 67–79. Basel: Karger.

CLYMAN, R.I., MANGANIELLO, V.C., LOVELL-SMITH, C.J. & VAUGHAN, M. (1976). Calcium uptake by subcellular fractions of human umbilical artery. *Am. J. Physiol.*, **231**, 1074–1081.

KLINNER, U., EHLERS, D., FERMUM, R. & MEISEL, P. (1977). Versuche zum Wirkungsmechanismus von Gefäßspasmolytika. 4. Wirkung von Nitroprussid-Natrium, Nitroglycerin, Prenylamin und Verapamil auf die Ca-Aufnahme von Mikrosomen aus glatter Gefäßmuskulatur. *Acta biol. med. germ.*, **36**, 1143–1148.

KREYE, V.A.W., BARON, G.D., LÜTH, J.B. & SCHMIDT-GAYK, H. (1975). Mode of action of sodium nitroprusside on vascular smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **288**, 381–402.

KREYE, V.A.W. & SCHLICKER, E. (1979). Effect of vasodilator drugs on the ^{45}Ca uptake of microsomes prepared from human umbilical arteries. *Naunyn-Schmiedeberg's Arch. Pharmac.*, Suppl. to **307**, R 42.

KREYE, V.A.W. & SCHLICKER, E. (1980). Effect of vasodilator drugs and alkaline phosphatase on ^{45}Ca uptake of sarcolemmal microsomes prepared from human umbilical arteries. In *Vascular Neuroeffector Mechanisms*. ed. Bevan, J.A., Godfraind, T.G., Maxwell, R.A. & Vanhoutte, P.M. pp. 4–6. New York: Raven Press.

KWAN, C.-Y., BELBECK, L. & DANIEL, E.E. (1979). Abnormal biochemistry of vascular smooth muscle plasma membrane as an important factor in the initiation and maintenance of hypertension in rats. *Blood Vessels*, **16**, 259–268.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

NISHIKORI, K., TAKENAKA, T. & MAENO, H. (1977). Stimulation of microsomal calcium uptake and protein phosphorylation by adenosine cyclic 3',5'-monophosphate in rat uterus. *Mol. Pharmac.*, **13**, 671–678.

NONOMURA, Y. & EBASHI, S. (1975). Isolation and identification of smooth muscle contractile protein. In *Methods in Pharmacology*, Volume 3, *Smooth Muscle*, ed. Daniel, E.E. & Paton, D.M. pp. 141–162. New York and London: Plenum Press.

REUTER, H., BLAUSTEIN, M.P. & HAEUSLER, G. (1973). Na–Ca exchange and tension development in arterial smooth muscle. *Phil. Trans. R. Soc. Lond. B*, **256**, 87–94.

ROCKSTEIN, M. & HERRON, P.W. (1951). Colorimetric determination of inorganic phosphate in microgram quantities. *Anal. Chem.*, **23**, 1500–1501.

THORENS, S. & HAEUSLER, G. (1978). Effects of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate on calcium uptake and phosphorylation in membrane fractions of vascular smooth muscle. *Biochim. biophys. Acta*, **512**, 415–428.

THORENS, S. & HAEUSLER, G. (1979). Effect of some vasodilators on calcium translocation in intact and fractionated vascular smooth muscle. *Eur. J. Pharmac.*, **54**, 79–91.

WEI, J.W., JANIS, R.A. & DANIEL, E.E. (1976). Isolation and characterization of plasma membrane from rat mesenteric arteries. *Blood Vessels*, **13**, 279–292.

WUYTACK, F., LANDON, E., FLEISCHER, S. & HARDMAN, J.G. (1978). The calcium accumulation in a microsomal fraction from porcine coronary artery smooth muscle. A study of the heterogeneity of the fraction. *Biochim. biophys. Acta*, **540**, 253–269.

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