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MEMBRANE ADENOSINE TRIPHOSPHATASE ACTIVITY OF VASCULAR SMOOTH MUSCLE

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Abstract—Differential centrifugation of rabbit aorta homogenates prepared in 0·25 M sucrose–30 mM histidine provided heavy, intermediate and light fractions. The light (microsomal) fraction was characterized by low cytochrome c oxidase, malate dehydrogenase and NADPH cytochrome c reductase activities and high NADH cytochrome c reductase and adenosine triphosphatase (ATPase) activities. Microsomal MgATPase differed from large granule MgATPase in the response to desoxycholate, Triton X-100, chlorpromazine, oligomycin and 2,4-dinitrophenol. The microsomal fraction contained significant amounts of guanosine and inosine triphosphatase activity, inhibited by Triton X-100. Mitochondrial and microsomal adenosine triphosphatase activity was stimulated by sodium hydrosulfite. Thiol inhibitors were without effect on microsomal adenosine triphosphatase activity. No monovalent cation dependence could be demonstrated, even in the presence of azide. These observations are discussed in reference to the membrane localization of microsomal MgATPase, the nature of aortic smooth muscle light microsomal fractions and the significance of such enzyme activity in monovalent cation transport at the smooth muscle membrane.

THERE IS considerable evidence that an adenosine triphosphatase (ATPase) participates in the transport or translocation of Na^+ and K^+ across cell membranes.[1] The role of providing energy for the active transport of Na^+ and K^+ and in controlling cellular permeability has been attributed to a [$Na^+ + K^+$] MgATPase. These conclusions have been obtained from studies conducted on nerve, striated muscle, brain, kidney and red cells in which there is parallel inhibition of active Na^+ transport and ATPase by cardiac glycosides[2,3] and where ATPase activity was dependent upon Na^+ and K^+ ions.[4] The enzyme system is located in a microsomal fraction which can be isolated by differential centrifugation.

The exact role of such an enzymatic mechanism in the maintenance of electrochemical gradients across smooth muscle membranes and their changes during activity is unclear. In fact, the role of electrochemical events in smooth muscle membrane and particularly in vascular muscle is under active discussion. Recent reviewers of the electrophysiology of smooth muscle have suggested that membrane spike activity is an obligatory component of the contractile event.[5] However, studies have shown that some vascular smooth muscle will contract without significant membrane potential changes[6] and that the depolarization-contraction sequence may be dissociated.[7–9] It seems likely that certain vasoactive drugs and possibly the neuroeffector humoral mechanism can activate some vascular muscle directly (pharmacomechanical coupling) without a casually important increase in selective permeability to Na^+ or K^+ ions.

Because of these considerations, it seemed desirable to study microsomal ATPase activity in vascular smooth muscle, especially that in which some of the previous observations pertaining to electrical-mechanical dissociation have been made. Emphasis in this discussion has been given to the identification and characterization of aortic microsomal ATPase and the determination of the magnitude of the [$Na^+ + K^+$] cardiac glycoside-sensitive component.

MATERIALS AND METHODS

Reagent grade chemicals were used unless otherwise specified. *p*-Chloromercuribenzoate (*p*CMB), reduced glutathione, NADH, ATP, ITP, UTP, GTP and ouabain were purchased from CalBiochem, Los Angeles, Calif. Chlorpromazine and *o*-iodobenzoic acid were purchased from K & K Laboratories, Hollywood, Calif., and cytochrome c was purchased from Mann Research, New York, N.Y. Atebrine was obtained from Winthrop Laboratories, New York, N.Y.

Preparation of tissues and ultracentrifugation. Adult rabbits, 2·1–2·6 kg were killed by cervical dislocation and rapidly bled. The thoracic aorta (300–450 mg wet wt.) was removed and placed in ice-cold 0·25 M sucrose–30 mM histidine, pH 7·6. The adventitial fat and connective tissue were removed extensively in the cold room at 4° and then cut into small pieces with cold scissors. Equal portions of crude homogenate were transferred to 2 all-glass Potter–Elvehjem homogenizers and made up to 4 ml prior to homogenization for 10 min in the cold. Homogenates were placed in 5-ml cellulose acetate tubes and centrifuged at 3000 g for 10 min in a Spinco model L ultracentrifuge with swinging rotor SW 39, $R_{max} = 9{\cdot}8$ cm. The supernatant (S_1) was centrifuged at 12,000 *g* for 10 min providing a heavy granule fraction (H). The supernatant was spun for 30 min at 50,000 *g* to give the intermediate fraction (I). The supernatant was recentrifuged at 102,000 *g* for 60 min and the resulting light microsomal fraction (L) was separated from cell sap (S_2).

Reference enzymes. Cytochrome c oxidase was assayed by a modification of the spectrophotometric method of Cooperstein and Lazarow,[10] activity being expressed as ΔE_{550}/min/mg protein. Malate dehydrogenase was measured by the spectrophotometric method of Bergmeyer and Bernt[11] in which the decrease in absorbance at 340 mμ was a measure of the utilization of NADH. Specific activity is expressed as ΔE_{340}/min/mg protein. NADH and NADPH cytochrome c reductases were measured by the method of Ernster *et al.*[12] Glucose 6-phosphatase was assayed in 0·1 M citrate buffer, pH 6·5, containing 0·08 M glucose 6-phosphate. Immediately after addition of 0·25 ml enzyme to 0·8 ml of the incubation system, 0·25 ml was removed and added to an equal volume of cold $(NH_4)_2SO_4$. After incubation for 10 min at 37°, a further 0·25 ml was withdrawn from the incubation system. After centrifugation for 3 min at 29,000 rpm, the inorganic phosphate in the supernatants was determined (see below) and enzyme activity was expressed as micro-moles $P_i(t_{10} - t_0)$/min/mg protein.

Assay of adenosine and other nucleoside triphosphatases. The incubation system (1 ml) contained 0·06 M Tris-HCl buffer (pH 8·1), 2 mM $MgCl_2$, 2 mM disodium ATP or appropriate nucleoside and 0·2 ml enzyme. Immediately after addition of enzyme, 0·25 ml was removed and placed in an equal aliquot of cold, saturated $(NH_4)SO_4$ in0·2 M sodium acetate, pH 4·0. After incubation at 37° for 10–15 min, a further

0·25 ml was removed and placed in saturated $(NH_4)_2SO_4$. The liberated inorganic phosphate was determined in the supernatant after centrifugation.

Determination of inorganic phosphate. A modification of the method of Peel and Loughman[13] was used in which aliquots of supernatant after $(NH_4)_2SO_4$ precipitation were added to 1 ml sodium acetate buffer, pH 4·0, containing approximately 5×10^{-6} M Cu^{2+}. An amount (0·2 ml) of 1% ascorbic acid, freshly prepared in 0·2 M sodium acetate buffer and 0·2 ml 1% ammonium molybdate in 0·1 N H_2SO_4, was added. The completed system remained at room temperature for 10 min prior to reading at 700 mμ in a Beckman DU spectrophotometer fitted with microcuvettes.

Protein was determined by the method of Lowry *et al.*[14] with crystalline bovine serum albumin as standard. Statistical analyses were performed according to Bernstein and Weatherall.[15]

RESULTS

Subcellular distribution of MgATPase in homogenates of rabbit aortic smooth muscle. Three particulate fractions were obtained from the ultracentrifugation of the supernatant, S_1, and were characterized with respect to total protein and total, specific, and relative specific activities of selected enzymes (Table 1 and Fig. 1). Over half the total protein was recovered in the cell sap, with an approximately equal distribution

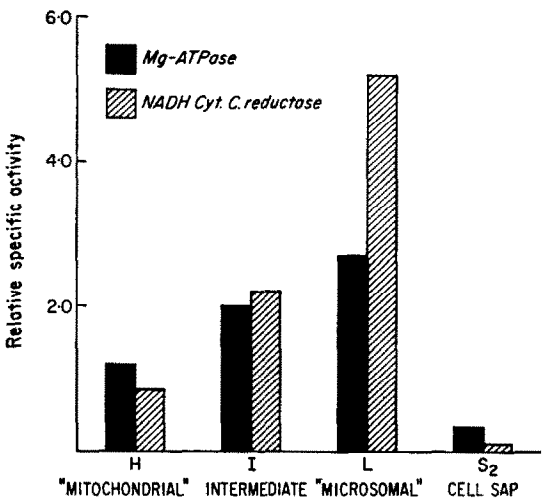

FIG. 1. Parallel comparison of relative specific activities of MgATPase and NADH cytochrome c reductase in 3 particulate fractions and in cell sap after the differential centrifugation of aortic smooth muscle homogenates prepared in 0·25 M sucrose–histidine. Values were calculated from mean values of specific activities. Relative specific activity is defined as: per cent total fractional enzyme activity/per cent total fractional protein.

of remaining protein in the three particulate fractions. The L ("microsomal") fraction contained less than 10 per cent of the cytochrome c oxidase; 40 and 28 per cent were recovered in the H and I fractions respectively. Malate dehydrogenase, an enzyme located in the mitochondrial matrix, was recovered in the cell sap (35 per cent) and H fraction (31 per cent); very little was found in the intermediate and microsomal fractions. This discrepancy between the mitochondrial marker enzyme, cytochrome

TABLE 1. TOTAL AND SPECIFIC ACTIVITIES (FIGURES IN PARENTHESES) OF SELECTED ENZYME VALUES IN PARTICULATE FRACTIONS OF AORTIC SMOOTH MUSCLE HOMOGENIZED IN 0·25 M SUCROSE–HISTIDINE*

Fraction (g–min)	Protein (mg)	Cytochrome c oxidase	Malate dehydro-genase	NADH cyt. c reductase	NADPH cyt. c reductase	Glucose 6-phosphatase	MgATPase
S_1	12·8 ± 0·37	0·45 ± 0·04	0·94	6·82 ± 0·28 (0·48)	0·86 ± 0·05 (0·062)	0·25	3·66 ± 0·11 (0·27)
H ($1·2 \times 10^5$)	1·63 ± 0·02	0·18 ± 0·03	0·30	0·71 ± 0·08 (0·40)	0·15 ± 0·02 (0·092)	~ 0·01	0·56 ± 0·02 (0·35)
I ($1·5 \times 10^6$)	2·22 ± 0·03	0·13 ± 0·03	0·09	2·64 ± 0·10 (0·99)	0·18 ± 0·04 (0·072)	~ 0·01	1·27 ± 0·04 (0·68)
L (6×10^5)	1·34 ± 0·03	0·04 ± 0·01	0·05	3·74 ± 0·17 (2·36)	0·12 ± 0·04 (0·076)	0·03	1·02 ± 0·05 (0·96)
S_2	7·30 ± 0·10	0·04 ± 0·0	0·35	0·05	0·42 ± 0·06 (0·055)	0·13	0·70 ± 0·03 (0·09)
Recovery (%)	97	89	85	106	108	~ 70	97

* Values (no. of observations, 4–7) are expressed as means ± S.E.M. See Methods for definition of enzyme units.

c oxidase, and malate dehydrogenase can be attributed to excessive rupture of mitochondria during the rigorous homogenization with loss and solubilization of malate dehydrogenase from ruptured mitochondria. Cytochrome c oxidase is membrane bound and the distribution of this enzyme marks the distribution of large and small mitochondria and mitochondrial membrane fragments. NADH cytochrome c reductase[12] and glucose 6-phosphatase[16] have been established as microsomal membrane-bound enzymes in liver preparations. A predominant localization of NADH cytochrome c reductase in the microsomal fraction was obtained, although a significant amount was present also in the intermediate fraction. No doubt this fraction contains an admixture of large "microsomal" membrane fragments and mitochondria. The low activities of glucose 6-phosphatase in all subfractions and the low recovery of this enzyme do not permit a critical evaluation.

Mg^{2+}-activated ATPase is bound to a membranous component of mitochondria and endoplasmic reticulum in liver.[12,17] The localization of MgATPase in all granule fractions of aortic smooth muscle homogenates agrees with the findings in other tissues and provides the initial evidence for the existence of a membrane MgATPase with high specific activity in the microsomal fraction of this tissue. The parallel changes in relative specific activities of NADH cytochrome c reductase and MgATPase (Fig. 1) suggest a close association of these two enzyme systems in a similar membrane fraction.

NADPH cytochrome c reductase was recovered primarily in the soluble cell sap, with an equal distribution of bound enzyme existing in the three particulate fractions. Moreover, in accord with observations of others,[18] the specific activity of NADH cytochrome c reductase in the light microsomal fraction was considerably greater than that of NADPH cytochrome c reductase.

Characterization and comparison of microsomal and mitochondrial MgATPase in aortic smooth muscle. The ATPase activity of aortic smooth muscle microsomal and mitochondrial fractions was dependent upon Mg^{2+} (Fig. 2). Stoichiometric concentrations of ATP and Mg^{2+} were optimal for adenosine triphosphatase activity in both fractions and such activity was linearly proportional to protein concentration. A single optimum pH peak (7·9–8·1) in Tris-HCl buffer characterized both mitochondrial and microsomal MgATPase activity. The apoenzyme in both fractions showed a greater affinity for Mg^{2+} than for Ca^{2+} at equimolar concentrations. Adenosine triphosphatase activities were stimulated (Fig. 3) to differing extent by increasing $[Ca^{2+}]$ in the absence of Mg^{2+} whereas Ca^{2+} added to the incubation system in the presence of 2 mM Mg^{2+} inhibited microsomal and mitochondrial activity approximately to the same degree.

A stimulation of rat liver mitochondrial and microsomal MgATPase by $Na_2S_2O_4$ has been reported.[19, 20] Stimulation was evident also in large granule and microsomal MgATPase prepared from aortic smooth muscle (Fig. 4). A greater percentage stimulation was noted with mitochondrial MgATPase, while considerable inhibition (Lineweaver–Burk plot) at concentrations greater than 5 mM sodium hydrosulfite was seen with microsomal MgATPase.

In a further comparison of microsomal and mitochondrial MgATPase activity (Table 2), the effects of azide, atebrine, chlorpromazine, 2,4-dinitrophenol and oligomycin were determined. Approximately similar degrees of inhibition were found in the two fractions in the presence of 5 mM azide, and 5 mM atebrine, but not with

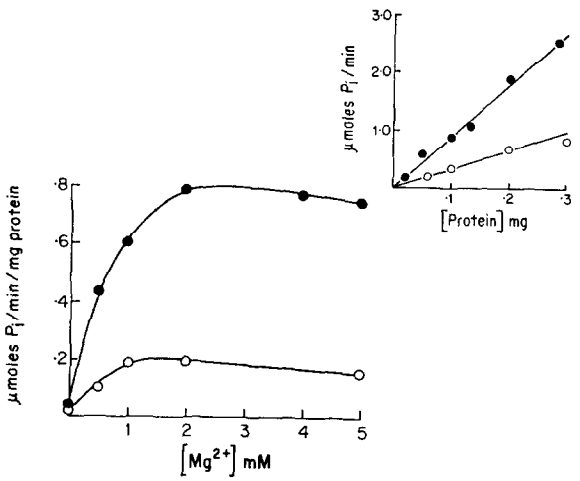

FIG. 2. Activation of aortic smooth muscle light microsomal (●—●) and mitochondrial (○—○) adenosine triphosphatase by Mg^{2+}. The insert reveals the strict linearity of MgATPase activity as a function of enzyme protein concentration. The assay contains 0·06 M Tris-HCl, pH 8·1; 2 mM disodium ATP; Mg^{2+}; and 0·2 ml enzyme.

chlorpromazine and oligomycin. The latter inhibited mitochondrial MgATPase approximately 50 per cent, while chlorpromazine preferentially inhibited the microsomal MgATPase. Lardy[21] showed oligomycin to act as a strong inhibitor of liver mitochondrial ATPase, while microsomal ATPase was unaffected. The approximately 50 per cent inhibition of microsomal MgATPase by 5 mM azide agrees well with that found with liver microsomal ATPase.[20] We obtained 25 per cent activation of mitochondrial MgATPase in the presence of 2,4-dinitrophenol but no significant change in microsomal enzyme activity.

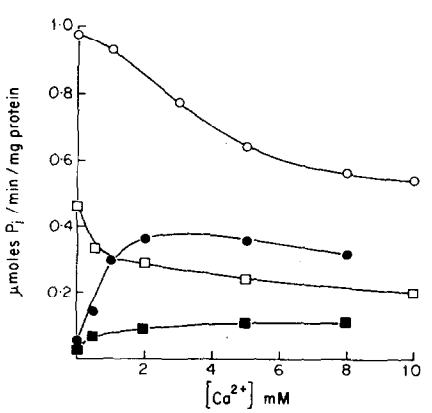

FIG. 3. Activation of aortic microsomal (●—●) and mitochondrial (■—■) adenosine triphosphatase by Ca^{2+}. Specific activities may be compared to the degree of activation found with Mg^{2+} (see Fig. 2). The remaining curves show inhibition of microsomal (○—○) and mitochondrial (□—□) MgATPase by Ca^{2+} at constant 2 mM Mg^{2+}. Assay conditions are as in Fig. 2.

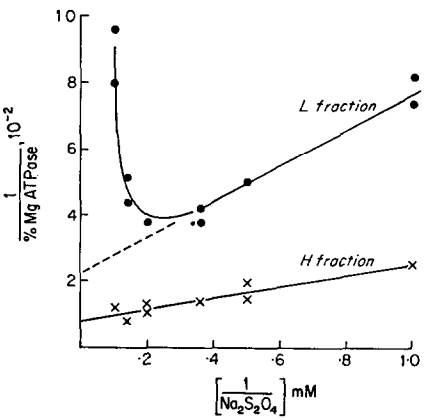

FIG. 4. Comparative Lineweaver–Burk plot of percentage stimulation of aortic microsomal (L fraction) and mitochondrial (H fraction) MgATPase by sodium hydrosulfite. Inhibition of microsomal activity is seen at $Na_2S_2O_4$ concentrations greater than 5 mM. Values are obtained from 2 experiments.

Effect of Triton X-100 and desoxycholate on nucleoside triphosphatase activity. In Table 3, the differential sensitivity of mitochondrial and microsomal MgATPase to equal concentrations of disrupting agent is shown. Marked inhibition of microsomal MgATPase was found at all concentrations of Triton X-100, but only at the maximal desoxycholate concentration of 0·3 per cent. No significant loss of enzyme activity was noted in the mitochondrial system with Triton X-100, but slight inhibition was noted with 0·3% (v/v) desoxycholate. This degree of inhibition may be secondary to binding of free Mg^{2+} by high concentrations of desoxycholate in the incubation system.[22]

Ernster and Jones[20] demonstrated a difference in the response of liver microsomal nucleoside triphosphatases to desoxycholate. Analogous studies on mitochondrial and microsomal nucleoside triphosphatases of aortic smooth muscle revealed different

TABLE 2. EFFECT OF AZIDE, ATEBRINE, CHLORPROMAZINE, OLIGOMYCIN AND 2,4-DINITROPHENOL ON MICROSOMAL AND LARGE GRANULE FRACTION MGATPASE FROM AORTIC SMOOTH MUSCLE*

Substance	Concn	MgATPase (μmoles P_i/min/mg protein) Microsomal	Large granule
Control		0·92	0·37
Azide	5 mM	0·52 (44)	0·13 (65)
Atebrine	5 mM	0·31 (66)	0·09 (76)
Chlorpromazine†	0·8 mM	0·27 (54)	0·23 (15)
Oligomycin	1 μg/ml	0·80 (13)	0·19 (49)
2,4-Dinitrophenol	1 mM	0·90 (2)	0·46 (+ 25)
Carnosine	2 mM	(+ 6)	

* Per cent inhibition in parentheses.

† Corrected readings for percentage inhibition of controls due to dissolving chlorpromazine in *N*, *N*-dimethylformamide: large granule fraction, 0·26; microsomal, 0·59.

degrees of activation and inhibition as a function of Triton X-100 concentration (Fig. 5). Guanosine, inosine and adenosine triphosphatase activity of the mitochondrial fraction varied between no change and approximately 100 per cent activation at Triton X-100 concentrations of 0·1–0·30 per cent inclusive. At similar concentrations, all nucleoside activities of the microsomal fraction were inhibited.

TABLE 3. EFFECT OF PREINCUBATION FOR 10 min AT 2° IN TRITON X-100 OR DESOXYCHOLATE (v/v) PRIOR TO TRANSFER TO ASSAY SYSTEM: 0·1 M TRIS-HCl, pH 8·1, CONTAINING 2 mM $MgCl_2$ AND 2 mM NaATP*

	P_i liberated (μmoles/min/mg protein)				
		Desoxycholate		Triton X-100	
Subfraction	Control	(0·1%)	(0·3%)	(0·1%)	(0·3%)
Mitochondrial	0·375	0·424	0·290	0·341	0·372
Microsomal	0·901	0·853	0·283	0·158	0·163

* Values are means of 2–3 experiments.

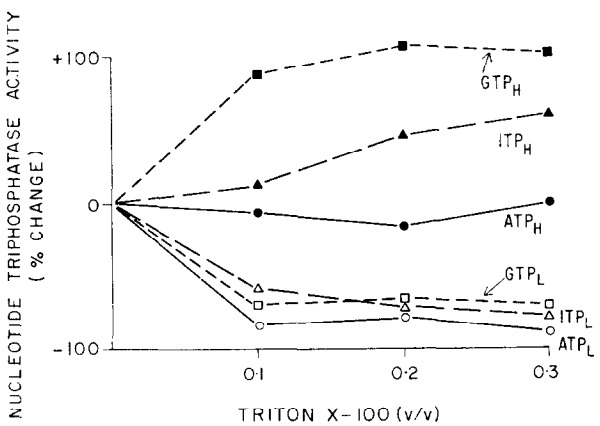

FIG. 5. Effect of Triton X-100 (nonionic detergent) on adenosine, guanosine and inosine triphosphatase activity of aortic microsomal (L) and mitochondrial (H) fractions. The assay was performed at pH 8·1, Tris-HCl buffer; Mg^{2+}, 2 mM; and nucleoside triphosphate, 2 mM.

Modification of microsomal MgATPase by [Na^+] *and* [K^+]. Attempts to demonstrate a [$Na^+ + K^+$]-sensitive component of aortic smooth muscle microsomal MgATPase were unsuccessful (Fig. 6). In these experiments, increasing K^+ concentrations to 100 mM failed to reveal MgATPase activation, and increasing Na^+ concentrations with or without K^+ further increased the slight inhibition seen. Ouabain, 5×10^{-5} M, failed to influence the activity of the enzyme in the presence of 20 mM K^+ + 80 mM Na^+. Azide has been shown to be a preferential inhibitor of microsomal MgATPase, while not affecting the [$Na^+ + K^+$] component.[23] A parallel plot of MgATPase and [$Na^+ + K^+$] MgATPase as a function of [azide] did not demonstrate potentiation of the cation-sensitive component (Fig. 7), which would be expected in the presence of low but significant amounts of [$Na^+ + K^+$] activity.

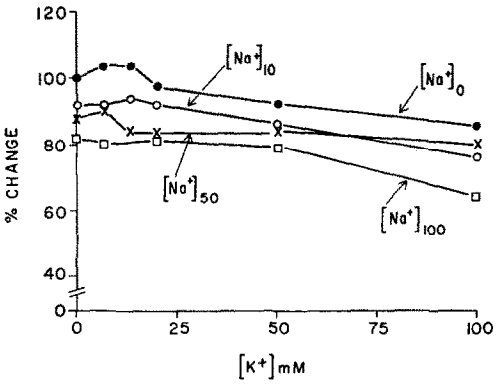

FIG. 6. Effect of Na^+ and K^+ on aortic smooth muscle microsomal MgATPase. Ions were present at indicated concentrations in the assay system, also containing 0·06 M Tris-HCl, pH 8·1; ATP, 2 mM; $MgCl_2$, 2 mM; and 0·2 ml enzyme. Results were obtained from 3 individual experiments

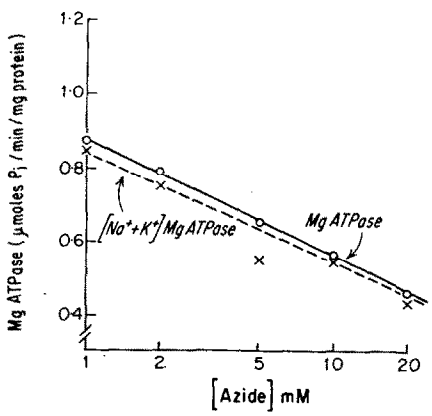

FIG. 7. Semilogarithmic plot of specific MgATPase activity in the absence (○—○) and presence (×- - -×) of (20 mM K^+ + 80 mM Na^+) as a function of sodium azide concentration. Assay conditions are similar to those in Fig. 6.

Effect of thiols and reducing agents on microsomal MgATPase activity. Thiol inhibitors, *p*CMB and iodobenzoate (0·1 mM), did not inhibit microsomal MgATPase activity (Table 4). Moreover, no stimulation of activity was observed in the presence of ascorbate, confirming the absence of free terminal reactive thiol groups in maintaining catalytic activity. Paradoxically, equimolar concentrations of L-cysteine and reduced glutathione inhibited the microsomal enzyme approximately 50 per cent. The reason for this is unclear, but L-cysteine and glutathione may have effectively removed Mg^{2+} from the assay system by chelation.

DISCUSSION

Significant ATPase activity was found in crude mitochondrial and microsomal fractions of aortic smooth muscle prepared by this particular centrifugation scheme. While there were numerous similarities between the two particulate enzyme systems—both were activated by Mg^{2+} and Ca^{2+}, stimulated by sodium hydrosulfite and inhibited by sodium azide and atebrine—differences were noted in their responsiveness

TABLE 4. EFFECT OF THIOL INHIBITORS AND REDUCING AGENTS ON AORTIC SMOOTH MUSCLE MICROSOMAL MgATPase*

Reagent	Concn (mM)	Sp. act. (μmoles P_i/min/mg protein)	Per cent change
Control		0·585	
Ascorbate	1·0	0·506	− 13
L-Cysteine	5·0	0·262	− 55
Reduced glutathione	5·0	0·182	− 68
p-CMB	0·1	0·535	− 8
Iodobenzoate	0·1	0·580	0

* Assay contains inhibitor, 2 mM Mg^{2+}, 2 mM ATP, Tris-HCl, pH 8·1. Values represent means of 2 experiments.

to oligomycin, in the preferential inhibition of microsomal MgATPase by Triton X-100 or desoxycholate and in the differing effects of Triton X-100 and desoxycholate on nucleoside triphosphatase activity in the two subfractions. Furthermore, 2,4-dinitrophenol was without effect on microsomal MgATPase, in contrast to the significant activation of mitochondrial ATPase.[24]

In view of the above differences observed between mitochondrial and microsomal MgATPase activity in vascular smooth muscle and the low cytochrome c oxidase activity in the microsomal fraction, it is unlikely that the "microsomal" fraction contains membrane fragments of mitochondrial origin. In the subfractionation of liver microsomes, Ernster *et al.*[12] demonstrated the close association of microsomal membrane with NADH-cytochrome c reductase, while NADPH-cytochrome c reductase was recovered in the supernatant. Our findings are consistent with these observations and provide evidence for the presence and membrane localization of a divalent cation-dependent ATPase in the microsomal fraction, distinct from mitochondrial ATPase.

The absence of $[Na^+ + K^+]$ stimulation suggests that the cation transport component of the MgATPase enzyme is absent in the microsomal system. This conclusion is supported by the lack of inhibition of MgATPase by ouabain. The enzyme activity thus differs fundamentally from $[Na^+ + K^+]$-linked MgATPase of other cell membranes isolated as a microsomal system.[25, 26] The slight inhibition of microsomal MgATPase observed after the simultaneous addition of the monovalent cations, Na^+ and K^+, may indicate the presence of a small $[Na^+ + K^+]$ MgATPase component.[27] However, azide is a preferential inhibitor of MgATPase[23] and would reveal a $[Na^+ + K^+]$ component if it were present. No such activity was demonstrated. It is apparent that aortic smooth muscle "microsomal" fractions do not contain a $[Na^+ + K^+]$ dependent MgATPase and are similar to microsomal fractions of liver and gastric mucosa in this respect.[20, 28] Such systems are usually not activated by Ca^{2+} alone; smooth muscle microsomal ATPase is activated by Ca^{2+} and Mg^{2+}, although the equimolar affinity for Mg^{2+} is greater than that for Ca^{2+}. Moreover, MgATPase is inhibited approximately 50 per cent by Ca^{2+} in the presence of Mg^{2+}.

A possible source of contamination in smooth muscle microsomal fractions could be derived from myosin ATPase. Such contamination is avoided partially by the introduction of a sucrose centrifugation system which allows for precipitation of

myosin and actomyosin ATPases in the preliminary spins. Moreover, only one pH optimum is present in contrast to two optima with myosin ATPase[29]; activation by Mg^{2+} is greater than that by Ca^{2+}; no reactive sulfhydryl groups are present; there is lack of activation by 2,4-dinitrophenol; activation by sodium hydrosulfite is highly significant and the decreasing nucleoside specificity of ATP > GTP > ITP is the converse of that found with actomyosin ATPase.[30] Microsomal MgATPase is markedly inhibited by desoxycholate and Triton X-100.

The membranous component associated with microsomal MgATPase is derived from the fragmented endoplasmic reticulum and cell membrane. Electron microscopic studies[31, 32] of the cell membrane in vascular smooth muscle revealed a complex, organized structure with multiple zones of micropinocytosis. This was in strong contrast to the paucity and uncomplicated development of the endoplasmic reticulum. Portman *et al.*,[33] from an examination of the phospholipid composition of subcellular fractions obtained from aortas, consider that the light microsomal fraction contains vesicles derived from plasmalemma and endoplasmic reticulum. Our observations are consistent with this interpretation. It is possible that the fraction is composed predominantly of micropinocytotic vesicles. A histoenzymatic association between ATPase activity and micropinocytotic vesicles has been reported.[34]

The absence of a $[Na^+ + K^+]$ component in vascular smooth muscle is perhaps not surprising in view of the limited or absent depolarization (Na^+ transport) occurring upon excitation[6] and is consistent with the pharmacomechanical coupling hypothesis for the activation of vascular smooth muscle.

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REFERENCES

1. J. C. Skou, *Physiol. Rev.* **45**, 596 (1965).
2. I. M. Glynn, *J. Physiol., Lond.* **136**, 148 (1957).
3. J. H. Schatzmann, *Helv. physiol. Pharmac. Acta* **11**, 346 (1953).
4. R. L. Post and P. C. Jolly, *Biochim. biophys. Acta* **25**, 119 (1957).
5. G. Burnstock, M. E. Holman and C. L. Prosser, *Physiol. Rev.* **43**, 482 (1963).
6. C. Su, J. A. Bevan and R. C. Ursillo, *Circulation Res.* **15**, 20 (1964).
7. W. H. Waugh, *Circulation Res.* **11**, 264 (1962).
8. J. A. Bevan, J. V. Osher and C. Su, *J. Pharmac. exp. Ther.* **139**, 216 (1963).
9. A. W. Cuthbert, in *Symposium on Electrical Activity and Innervation in Blood Vessels*, Vol. 8, Bibl. Anat. (1966).
10. S. J. Cooperstein and A. Lazarow, *J. biol. Chem.* **189**, 665 (1951).
11. H. U. Bergmeyer and E. Bernt, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 757. Academic Press, New York (1963).
12. L. Ernster, P. Siekevitz and G. E. Palade, *J. Cell Biol.* **15**, 541 (1962).
13. J. L. Peel and B. C. Loughman, *Biochem. J.* **65**, 709 (1957).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. L. Bernstein and M. Weatherall, *Statistics for Medical and Other Biological Students*, p. 74. E. & S. Livingstone, London (1952).
16. H. Beaufay, D. S. Bendall, P. Baudhuin and C. de Duve, *Biochem. J.* **73**, 623 (1959).
17. P. Siekevitz, H. Low, L. Ernster and O. Lindberg, *Biochim. biophys. Acta* **29**, 378 (1958).
18. B. Reynafarje and V. R. Potter, *Cancer Res.* **17**, 1112 (1957).
19. D. K. Myers and E. C. Slater, *Biochem. J.* **67**, 572 (1957).

21. H. LARDY, in *Biological Structure and Function* (Eds. T. W. GOODWIN and O. LINDBERG), p. 265. Academic Press, London (1961).
22. R. RENDI and T. HULTIN, *Expl Cell Res.* **18**, 542 (1959).
23. F. J. SAMAHA and J. GERGELY, *Archs Biochem. Biophys.* **109**, 75 (1965).
24. A. L. LEHNINGER, in *The Mitochondrion*, p. 92. W. A. Benjamin, New York (1965).
25. U. MUSCATELLO, E. ANDERSSON-CEDERGREN and G. F. AZZONE, *Biochim. biophys. Acta* **63**, 55. (1962).
26. J. JARNEFELT, *Biochim. biophys. Acta* **59**, 643 (1962).
27. J. B. MODOLELL and R. O. MOORE, *Biochim. biophys. Acta* **135**, 319 (1967).
28. D. K. KASBEKAR and R. P. DURBIN, *Biochim. biophys. Acta* **105**, 472 (1965).
29. W. F. H. M. MOMMAERTS and I. GREEN, *J. biol. Chem.* **208**, 833 (1954).
30. W. HASSELBACH, *Biochim. biophys. Acta* **25**, 365 (1957).
31. F. PARKER and G. F. ODLAND, *Am. J. Path.* **48**, 197 (1966).
32. M. A. VERITY and J. A. BEVAN, *J. Anat.* **103**, 49 (1968).
33. O. W. PORTMAN, M. ALEXANDER and C. A. MARUFFO, *Archs Biochem. Biophys.* **122**, 344 (1967).
34. V. T. MARCHESI and R. F. BARNETT, *J. Ultrastruct. Res.* **10**, 10 (1964).