# Inhibition of Muscle Cell Membrane ATPase by Quinidine.* (28902)

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The adenosinetriphosphatase (ATPase) activities of skeletal muscle granules and myofibrils have been shown to be inhibited by quinidine(1). Recent evidence of a possible correlation between cell membrane ion transport and membrane ATPase(2), together with suggestions that quinidine may have a role in regulation of membrane permeability(3-5), led us to study the effect of quinidine on ATPase activity of skeletal muscle membranes.

*Materials and methods.* Cell membranes were prepared from hind leg and back muscles of albino rats according to the procedure described by Kono and Colowick(6). Membranes were used at the first stage of purification; *i.e.,* after precipitation from 1 M KCl. Unless otherwise stated, ATPase was measured in a system containing 200 $\mu$moles tris(hydroxymethyl) aminomethane, pH 7.4, 100 $\mu$moles $Na^+$, 50 $\mu$moles $K^+$, 10 $\mu$moles $Mg^{++}$, and 10 $\mu$moles adenosinetriphosphate, pH 7.4 in a total volume of 2 ml. After incubation for 15 minutes at 37°C the reaction was terminated by adding 0.2 ml of 10 N $H_2SO_4$. In order to adsorb quinidine (which interferes with determination of inorganic phosphate) and nucleotide, 200 mg of activated charcoal (Norit A) was added, the tubes diluted to 10 ml and filtered after 10 minutes. Inorganic phosphate was determined in aliquots of the filtrate by the method of Fiske and Subbarow(7). Appropriate controls and standards were run with each set of assays. Membrane protein was determined by the method of Lowry(8) using gelatin as a standard(6).

*Results and discussion.* For hydrolysis of adenosinetriphosphate by membranes both sodium and magnesium must be present in the reaction mixture indicating the possible identity of the muscle membrane ATPase and the Na-K activated ATPase described in neuron and erythrocyte membranes(2). However, in these experiments with muscle membranes, it was not established that potassium is required for activity inasmuch as the membranes are prepared in KCl solution. The membrane ATPase is somewhat labile (Table I), more than 75% of the activity disappearing after storage of membrane suspensions at 4°C for 7 days.

Quinidine inhibits this ATPase (Table I). The mean inhibition of ATP hydrolysis in the presence of $5 \times 10^{-4}$M quinidine sulfate is 45%. Membrane APTase is somewhat more sensitive to quinidine than is the myofibrillar or granular ATPase of muscle (about 20% inhibition at $5 \times 10^{-4}$M quinidine sulfate) but less sensitive than the latent ATPase of rat liver mitochondria (38% at $1 \times 10^{-5}$M quinidine sulfate)(1). The inhibition of membrane ATPase was a constant percentage of the ATPase activity at several enzyme and substrate concentrations. Quinidine inhibition is maximal at optimum magnesium concentrations (Table II) but at concentrations where magnesium becomes inhibitory, the effect of quinidine is decreased, suggesting that high concentrations of magnesium and quinidine may inhibit at the same enzymic site. The same sort of relationship between quinidine and calcium inhibition of muscle granular ATPase was found(1).

If membrane ion transfer depends in some way on the hydrolysis of adenosinetriphosphate by the membrane ATPase, as appears likely(2), the effect of quinidine on membrane ion permeability(4,5) may be due to quinidine inhibition of this enzyme. The range of concentrations of quinidine sulfate which produce inhibition ($5 \times 10^{-5}$M to $5 \times 10^{-4}$M) is not attained in blood following administration of quinidine in therapeutic doses(9,10). However, quinidine is concentrated in a number of tissues including skele-

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TABLE I. Inhibition of Skeletal Muscle Cell Membrane Adenosinetriphosphatase by Quinidine. Figures are μmoles of inorganic phosphate formed in 15 minutes per mg of membrane protein. Figures in parentheses are percent inhibition.

| Membrane preparation | Age* | Concentration of quinidine (M) None | $1 \times 10^{-5}$ | $5 \times 10^{-5}$ | $1 \times 10^{-4}$ | $5 \times 10^{-4}$ |
|---|---|---|---|---|---|---|
| 1 | 24 hr | 1.04 | — | — | — | .52 (50) |
| 2 | Fresh | 1.38 | — | — | — | .79 (43) |
| " | 24 hr | 1.04 | — | — | — | .58 (44) |
| 3 | Fresh | 1.27 | 1.27 (0) | 1.12 (11) | .98 (23) | .68 (46) |
| 4 | " | 1.57 | 1.57 (0) | 1.46 (7) | 1.31 (17) | .90 (43) |
| " | 7 days | .39 | — | — | — | — |

* Hours or days at 4°C. Fresh means experiment was performed within 4 hr after preparing membranes.

tal muscle and may reach a concentration above $1 \times 10^{-4}$M in myocardium (calculated from the data of Scherlis *et al*) (10).

*Summary.* The adenosinetriphosphatase activity of rat skeletal muscle membranes is inhibited about 45% by $5 \times 10^{-4}$M quinidine sulfate and slightly (about 9%) by $5 \times 10^{-5}$ M quinidine sulfate. This enzymic activity requires sodium and magnesium. Higher concentrations of magnesium are inhibitory and the inhibitory effect of quinidine is decreased at high magnesium concentrations.

TABLE II. Quinidine Inhibition of Membrane Adenosinetriphosphatase at Various Magnesium Concentrations. Figures are as in Table I.

| $Mg^{++}$ added to reaction (M) | Adenosinetriphosphatase activity: No quinidine | $5 \times 10^{-4}$M quinidine | Inhibition (%) |
|---|---|---|---|
| None | .13 | .10 | 23 |
| .0005 | .66 | .40 | 39 |
| .0025 | 1.37 | .72 | 47 |
| .005 | 1.41 | .81 | 43 |
| .010 | .97 | .62 | 36 |
| .015 | .68 | .49 | 28 |

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## Influence of a Synthetic Analogue of Vasopressin on Survival After Hemorrhagic Shock in Rats.* (28903)

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The value of vasoexcitor-pressor drugs in treating shock is being vigorously questioned (1,2) primarily from experience with the amine pressors. The critical disadvantage of the latter is attributed principally to tissue ischemia resulting from their intense constrictor effects on the resistance arterioles. Little therapeutic distinction has been made between the unfavorable constrictor effects of

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