# ATPase AND ADENYLATE CYCLASE DISTRIBUTION IN SKELETAL MUSCLE MEMBRANES

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## 1. Introduction

The action of most hormones is initiated by binding and formation of a hormone–receptor complex. The receptors for the water-soluble hormones are located in the target cell plasma membrane which enables recognition and binding of the hormone from the extracellular fluid. Epinephrine and glucagon increase intracellular levels of cAMP by stimulating the enzyme adenylate cyclase. A physical association, termed coupling, between the hormone receptor complex and adenylate cyclase necessitates the presence of adenylate cyclase in the plasma membrane as well as the receptor. The possibility that adenylate cyclase also exists in the internal membrane systems of the target cell has received little attention.

Cheng and Farquhar investigated the subcellular distribution of adenylate cyclase in rat liver tissue [1]. Specific activities for basal and NaF-activated enzyme were 10-fold greater in Golgi membrane than plasma membrane. Significantly, glucagon stimulated adenylate cyclase was detected almost exclusively in plasma membrane consistent with the presence of a coupled, glucagon receptor–adenylate cyclase system. Further cytochemical evidence suggested that contamination of membrane fractions had not occurred [2].

Adenylate cyclase activity has been reported in isolated sarcoplasmic reticulum (microsomes) of striated muscle [3–5]. These studies did not include equivalent data on isolated sarcolemma (plasma membrane) making distribution comparisons difficult. Here, enriched fractions of sarcolemma and sarcoplasmic reticulum were identified on the basis of relative $Na^+,K^+$ ($Mg^{2+}$)-ATPase and $Ca^{2+},Mg^{2+}$-ATPase activities [6,7]. Membranes were prepared and studied from rabbit and rat skeletal muscle. It is shown that epinephrine-stimulated adenylate cyclase activity is 3–5-fold greater in sarcolemma compared to sarcoplasmic reticulum while basal and NaF-activated enzyme activities are equivalent.

## 2. Materials and methods

Enriched fractions of sarcolemma and sarcoplasmic reticulum were prepared from the lower limb skeletal muscle of the New Zealand White Rabbit. The LiBr extraction procedure used for rat muscle membrane isolation was employed with the following modifications [8]. Finely minced muscle, 10 g in 10 vol. (w/v) sucrose buffer (0.25 M sucrose, 0.1 M Tris–HCl, 1 mM $Na_2$ EDTA (pH 7.6)) was disrupted by Polytron homogenization (setting 7.5, 15 s) at 4°C. The homogenate was centrifuged at 4000 × *g* for 10 min to separate the nuclear pellet (NP) from the mitochondrial supernatant. The latter fraction was centrifuged at 9750 × *g* for 10 min to obtain a microsomal supernatant; the mitochondrial pellet was discarded. The NP and microsomal fraction (MF) were extracted for 14 h at 4°C in LiBr medium (0.5 M LiBr, 0.1 M Tris–HBr, pH 8.5). The subsequent differential centrifugation and KCl wash steps were identical to [8]. Crude membrane from the initial NP and MF was applied to continuous sucrose density (15–35%) gradients and centrifuged at 200 000 × *g* for 210 min in an SW-41 swinging bucket rotor (Beckman L5-50 ultracentrifuge).

ATPase activity was determined at 37°C in 1.0 ml final vol. consisting of 50 μg membrane protein, 30 mM glycylglycine, 30 mM imidazole, 0.75 mM $H_4$EDTA, 1 mM Tris–ATP (pH 7.5). $Na^+,K^+$ ($Mg^{2+}$)-ATPase was measured in the presence of 100 mM NaCl, 20 mM KCl and 5 mM $MgCl_2$. Net $Na^+,K^+$

($Mg^{2+}$)-ATPase was determined by subtraction of activity in the presence of 5 mM $MgCl_2$ alone. $Ca^{2+}$-ATPase was measured at a final [$CaCl_2$] of 5 mM. Reaction time was 5 min. $P_i$ produced was measured by the method in [9].

Adenylate cyclase activity was determined at 30°C in 100 μl final vol. consisting of 50 μg membrane protein, 50 mM Tris–HCl, 10 mM $MgCl_2$, 20 mM creatine phosphate, 10 units creatine kinase (145 U/mg), 2 mM cAMP, 1 mM Tris–ATP, plus $1–5 \times 10^6$ cpm [$\alpha$-$^{32}$P]ATP (pH 7.5). Effectors at final concentration were 10 mM NaF and $1 \times 10^{-4}$ M epinephrine bitartrate. The reaction time was 5 min. Cyclic [$^{32}$P]AMP produced was measured by the method in [10]. Reaction rates were linear with respect to time and protein concentration. The concentration of effectors was such to give maximal enzyme stimulation.

Protein content of membrane subfractions was determined by the Lowry method using bovine serum albumin as standard [11].

[$\alpha$-$^{32}$P]ATP (10–30 Ci/mmol) was purchased from New England Nuclear. Tris–ATP, phosphocreatine and creatine kinase were from Sigma Chem. Co.

## 3. Results

The two major membrane fractions demonstrated distinctly different distributions in sucrose density gradients (fig.1). Material originating from the NP could be differentiated from the MF by a symmetric peak in the 18–27% [sucrose] range of the gradient. For analysis of ATPase and adenylate cyclase activities each gradient was divided into subfractions corresponding to heavy (A), middle (B,C) and light (D) density zones.

ATPase activity in skeletal muscle shows a precise location related to specific membrane function. The $Na^+,K^+$ ($Mg^{2+}$)-ATPase is found in the sarcolemma where it regulates the maintenance of the resting membrane potential. The $Ca^{2+}$-ATPase resides principally in the sarcoplasmic reticulum reflecting the $Ca^{2+}$-transport activity of this membrane system in the relaxation phase of the contractile cycle [12]. In the present study, therefore, $Na^+,K^+$ ($Mg^{2+}$)-ATPase and $Ca^{2+}$-ATPase were measured with adenylate cyclase in an attempt to define the distribution of adenylate cyclase between sarcolemma and sarcoplasmic reticulum.

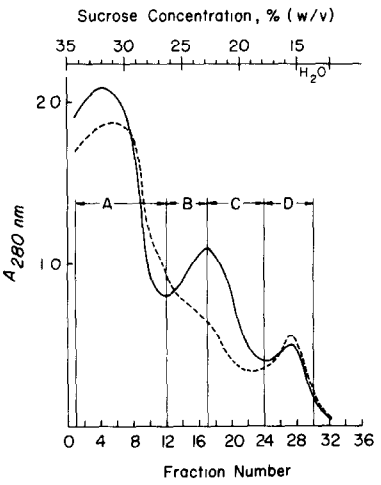


Fig.1. Membrane distribution after sucrose density gradient centrifugation. Membranes derived from the initial nuclear pellet (NP, solid line) and microsomal fraction (MF, dotted line) was divided into four subfractions (A–D) for determining the distribution of $Na^+,K^+$ ($Mg^{2+}$), $Mg^{2+}$,$Ca^{2+}$-ATPase and adenylate cyclase activity.

$Na^+,K^+$ ($Mg^{2+}$)-ATPase activity was found exclusively in membrane material derived from the original NP (fig.2). The highest specific activity (460 nmol $P_i \cdot min^{-1} \cdot mg^{-1}$) was in membrane subfraction C. In contrast, $Ca^{2+}$,$Mg^{2+}$-ATPase activity was 12–14-fold greater in membranes derived from MF (1300–1400 nmol $P_i \cdot min^{-1} \cdot mg^{-1}$) compared to NP (100–150 nmol $P_i \cdot min^{-1} \cdot mg^{-1}$). Virtually no $Na^+,K^+$ ($Mg^{2+}$)-

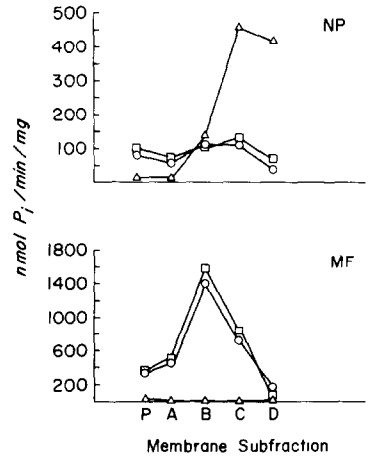


Fig.2. Distribution of ATPase activity in the NP (upper panel) and MF (lower panel). Net $Na^+,K^+$ ($Mg^{2+}$)-ATPase (△——△); $Mg^{2+}$-ATPase (○——○); $Ca^{2+}$-ATPase (□——□).

ATPase activity was detected in microsomal membranes. Thus, a clear separation of muscle membranes, determined by distribution of specific ion-stimulated ATPase activity, was achieved by the membrane isolation procedure. Based on ATPase function in muscle surface and internal membranes we assign NP as the source for enriched sarcolemma; MF for enriched sarcoplasmic reticulum.

Adenylate cyclase is considered to be a surface membrane enzyme. Basal and NaF stimulation of adenylate cyclase is direct and does not require the presence of a coupled hormone–receptor complex. These two activities are therefore used to assess 'total' enzyme activity in comparison to that portion stimulated by a given hormone. In our studies, basal activities (50 vs 65 pmol cAMP . $min^{-1}$ . $mg^{-1}$) and NaF-stimulated activities (600 vs 660 pmol cAMP . $min^{-1}$ . $mg^{-1}$) were not significantly different between NP- and MF-derived membranes (fig.2). Membranes possessing $Na^+,K^+$ ($Mg^{2+}$)-ATPase (NP) however, showed a 3-fold greater epinephrine-stimulated adenylate cyclase than MF membranes (290 vs 107 pmol cAMP . $min^{-1}$ . $mg^{-1}$).

$Na^+,K^+$ ($Mg^{2+}$)-ATPase, $Ca^{2+},Mg^{2+}$-ATPase and adenylate cyclase activities were also measured in membranes enriched for sarcolemma and sarcoplasmic reticulum isolated from rat skeletal muscle (table 1). Similar to the rabbit, rat muscle membranes derived from NP and MF displayed equivalent basal and NaF-activated adenylate cyclase activities. Membranes with the highest $Na^+,K^+$ ($Mg^{2+}$)-ATPase activity showed a 5-fold greater epinephrine-activated enzyme activity compared to sarcoplasmic reticulum (50.2 vs 11.8 pmol cAMP . $min^{-1}$ . $mg^{-1}$). $Ca^{2+},Mg^{2+}$-ATPase activity was 5-fold greater in membranes derived from sarcoplasmic reticulum compared to sarcolemma (2750 vs 517 nmol . $min^{-1}$ . $mg^{-1}$).

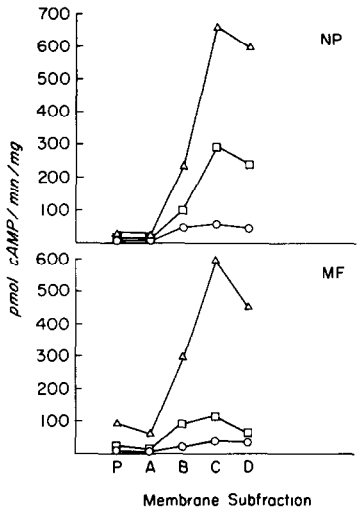


Fig.3. Distribution of adenylate cyclase activity in the NP (upper panel) and MF (lower panel). Basal (○——○); 10 mM NaF (△——△); 0.1 mM epinephrine (□——□).

## 4. Discussion

The results of this study demonstrate the presence of adenylate cyclase in skeletal muscle sarcoplasmic reticulum as well as sarcolemma. The major difference

Table 1

| Activity | Membrane fraction | |
|---|---|---|
| | Sarcolemma | Sarcoplasmic reticulum |
| 1. $Na^+,K^+$ ($Mg^{2+}$)-ATPase | 648 ± 48 | 25 ± 2.5 |
| $Ca^{2+}$-ATPase | 517 ± 100 | 2750 ± 367 |
| 2. Adenylate cyclase | | |
| (a) Basal | 17.8 ± 0.9 | 19.9 ± 2.6 |
| (b) 10 mM NaF | 321 ± 53 | 251 ± 26 |
| (c) 0.1 mM epinephrine | 50.2 ± 2.2 | 11.8 ± 3.9 |

Membranes were isolated from rat skeletal muscle as in section 2. $Na^+,K^+$ ($Mg^{2+}$)-ATPase activity is expressed as nmol $P_i$ . $min^{-1}$ . $mg^{-1}$. Adenylate cyclase activity is expressed as pmol cAMP . $min^{-1}$ . $mg^{-1}$. Values represent the mean ± SEM for 4 separate membrane preparations

in enzyme distribution became evident only when epinephrine stimulated adenylate cyclase was measured. Under this condition, membranes displaying the highest activity for $Na^+$,$K^+$ ($Mg^{2+}$)-ATPase also exhibited a 3–5-fold greater epinephrine-stimulated enzyme compared to sarcoplasmic reticulum. Muscle sarcolemma would be predicted to have the coupled, epinephrine receptor–adenylate cyclase system to enable the binding of epinephrine from the extracellular environment and stimulation of cAMP synthesis.

Adenylate cyclase activity in the sarcoplasmic reticulum supports claims for cardiac muscle sarcoplasmic reticulum activity [4,13,14]. cAMP-dependent protein phosphorylation of the sarcoplasmic reticulum was shown paralleled by the accumulation of $Ca^{2+}$ and stimulation of $Ca^{2+}$-ATPase [4,13,14]. Sarcoplasmic reticulum adenylate cyclase may respond to internal effector molecules, possibly $Ca^{2+}$ itself, to synthesize cAMP specifically for the regulation of sarcoplasmic reticulum protein kinase and the attendant $Ca^{2+}$ movements during muscle contraction.

Finally, caution must be exercised in using adenylate cyclase as a plasma membrane marker. The hormone stimulated enzyme and not simply the basal and/or NaF-activated enzyme should be measured for surface membrane identification studies.

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