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S-100b protein regulates the activity of skeletal muscle adenylate cyclase in vitro

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We have investigated the effect of the b isoform of S-100 proteins on adenylate cyclase activity of rat skeletal muscle. S-100b inhibits the adenylate cyclase activity in the presence of Mg^{2+} (5.0–50 mM), while it activates the same enzyme in the presence of Ca^{2+} (0.1–1.0 mM) dose-dependently in both cases. S-100b counteracts the stimulatory effect of NaF on adenylate cyclase in the presence of Mg^{2+} and the inhibitory effect of RMI 12330 A in the presence of Ca^{2+}.

Protein S-100; Adenylate cyclase; (Skeletal muscle)

1. INTRODUCTION

It is well established that the activity of adenylate cyclase (ATP pyrophosphate lyase cyclizing EC 4.6.1.1) is modulated by membrane proteins such as the G-proteins [1–5] as well as by cytoplasmic proteins. Among the latter, the ubiquitous intracellular Ca^{2+}-binding protein, calmodulin, has been shown to regulate a particular (calmodulin-sensitive) adenylate cyclase activity in the brain and other tissues [6,7]. Calmodulin stimulates the adenylate cyclase activity by interacting directly with the catalytic component of the enzyme [8].

A group of three 21 kDa, acidic, Ca^{2+}-binding proteins known as S-100 belongs to the family of Ca^{2+}-modulated, EF hand-type polypeptides that are structurally related to calmodulin and other acidic Ca^{2+}-binding proteins (see [9] for a recent review). S-100 is present in many cell types, although individual S-100 isoforms ($S\text{-}100a_o$, S-100a and S-100b) are differently distributed in cells [9–13]. S-100 proteins have been shown to regulate a number of kinase and phosphoprotein phosphatase activities [14], a brain aldolase activity [15] and the process of microtubule assembly-disassembly [16] in vitro. Some of these effects are strictly Ca^{2+}-dependent, while others are not. More recently, the human gene encoding the β-subunit of S-100 has been located on chromosome 21 and this has been related to some neurological aspects of Down syndrome [17].

In view of the structural similarity of S-100 to calmodulin, we sought to investigate the effects of S-100b protein on adenylate cyclase activity of rat skeletal muscle. The results indicate that S-100b inhibits the adenylate cyclase activity in the presence of Mg^{2+} and increases this activity in the presence of Ca^{2+}. Both effects are obtained using a concentration of S-100b in the range 0.05–5 μM.

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Abbreviation: RMI 12330 A, *N*-(*cis*-2-phenylcyclopentyl)-azacyclotridecan-2-imine hydrochloride

2. EXPERIMENTAL

S-100b was purified from rat brain and characterized as described [18]. TRK 432 cAMP assay kit was obtained from the radiochemical Center (Amersham, England). ATP, GTP, DTT

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and BSA were from Sigma (St. Louis, MO). RMI 12330 A [*N*-(*cis*-2-phenylcyclopentyl)-azacyclotridecan-2-imine hydrochloride] was a generous gift from Dr C. Biondi (Istituto di Fisiologia Generale, Università di Ferrara, Italy).

Skeletal muscles from adult rat legs were used. Samples (10 g) were minced, and homogenized in 50 mM Tris-maleate (pH 7.4) containing 2 mM EGTA. The homogenate was filtered through four layers of cheese-cloth and the residue resuspended in the same buffer at a final protein concentration of 2.5 mg/ml. The suspension was immediately used for the adenylate cyclase assay [19]. The assay medium (pH 7.4) consisted of the following (final concentrations): 50 mM Tris-acetate, 5 mM Teophylline, 5 mM DTT, 0.5 mg/ml BSA, 0.05 mM GTP (in some experiments 0.5 mM), 50–200 μg muscle proteins and H_2O to a final volume of 0.5 ml. After 5 min of incubation at 4°C, the test substances (S-100b, RMI, NaF) were added to the final concentrations reported in the figure and table legends. The incubation was continued for 10 min at 4°C then the reaction was started by adding 5 mM ATP and Me^{2+} (Mg^{2+} or Ca^{2+}), and continued during the next 10 min at 37°C in a shaking water bath. The reaction was stopped by boiling for 5 min. The samples were stored at 4°C overnight and microfuged (18 000 × *g* × 5 min) for precipitation of denatured proteins. The supernatants were collected and the cAMP determination was carried out by a displacement binding assay using a commercial kit. Protein was measured by the method of Lowry et al. against a standard solution of BSA [20].

3. RESULTS

Adenylate cyclase is weakly active in the absence of divalent cations such as Mg^{2+}, Ca^{2+} or Mn^{2+}. A crude preparation of the enzyme obtained from rat skeletal muscle has an activity of 6.5 pmol cAMP produced/mg protein per min (table 1). 5 mM Mg^{2+} increases this activity to a maximum of 165.6 pmol cAMP/mg protein per min with an approximate 30-fold activation (table 1).

Under these conditions, S-100b (5 μM) causes a reduction in the Mg^{2+}-activated activity to 20% of that detectable in the presence of Mg^{2+} alone (table 1).

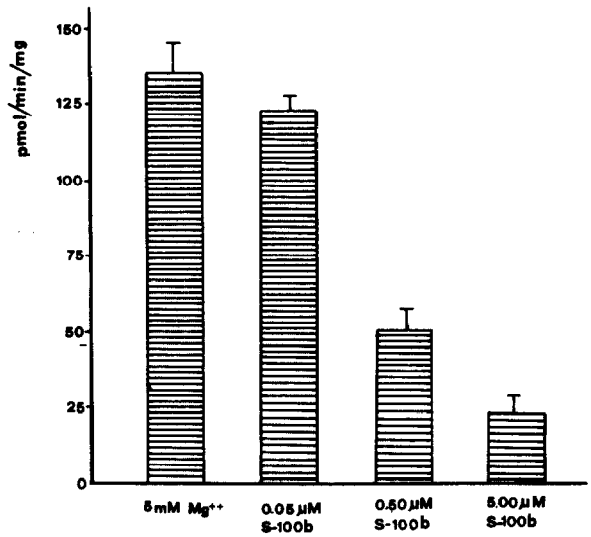

Fig.1. Effect of different concentrations of S-100b (0.05–5.0 μM) on adenylate cyclase activity measured in the presence of 5 mM Mg^{2+}. The data given refer to one of four experiments (mean ± SD, $n=5$).

In a total of five experiments performed with three different enzyme preparations, the inhibition exerted by 5 μM S-100b ranged between 70 and 80%. At 5 mM Mg^{2+} the inhibitory effect is dose-dependent and half-maximal between 0.05 and 0.5 μM S-100b (fig.1).

In a preliminary attempt to elucidate the mode of action of S-100b, we tested its effect in the presence of two substances known to activate (NaF) [21] or to inhibit (RMI) [22] adenylate cyclase. These substances have been shown to act on the in-

Table 1

Effect of 5 μM S-100b on adenylate cyclase activity in the presence of Mg^{2+}, NaF or the inhibitor RMI

	− S-100b	+ S-100b
None	6.5 ± 0.3	5.2 ± 0.2
5 mM Mg^{2+}	165.6 ± 14.2	46.2 ± 2.1
50 mM Mg^{2+}	241.3 ± 27.1	161.8 ± 14.7
5 mM Mg^{2+}/1 mM NaF	235.2 ± 31.0	145.7 ± 16.3
5 mM Mg^{2+}/1 mM RMI	12.8 ± 7.4	14.6 ± 5.8

The values are expressed as pmol/min per mg and represent the mean ± SD of nine measurements

Table 2

Effect of 5 μM S-100b on adenylate cyclase in the presence of Ca^{2+} and RMI

	− S-100b	+ S-100b
None	4.9 ± 0.2	4.6 ± 0.1
0.1 mM Ca^{2+}	57.6 ± 14.9	107.0 ± 13.4
1.0 mM Ca^{2+}	57.0 ± 10.8	104.0 ± 13.1
1.0 mM Ca^{2+}/1.0 mM RMI	16.8 ± 6.5	35.4 ± 7.0

The activity is expressed as pmol/min per mg and it is referred to mean ± SD of eleven measurements

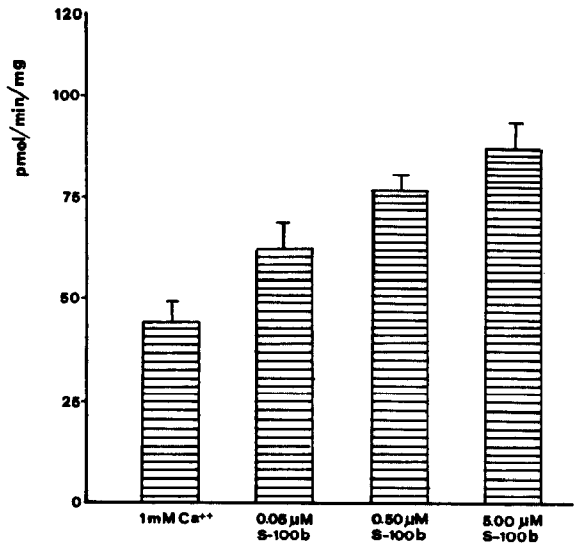

Fig.2. Activating effect of S-100b (0.05–5.0 μM) measured in the presence of 1 mM Ca^{2+}, on adenylate cyclase activity. Data reported are from one of three experiments carried out in quadruplicate.

termediate component of the enzyme complex. S-100b blocks the activating action of NaF and maintains the activity at the level detected when the enzyme is assayed without F^- (table 1). On the other hand, S-100b does not alter the inhibitory activity exerted by RMI (table 1).

Adenylate cyclase is also markedly stimulated by Ca^{2+} which shifts the activity from a basal level of 4.9–57.6 pmol/min per mg (table 2). S-100b (5 μM) increases the activating effect of Ca^{2+} by 100% (table 2) with a half-maximal effect between 0.05 and 0.5 μM (fig. 2). Since S-100b alone has no activating effect (tables 1 and 2), we conclude that S-100b amplifies the Ca^{2+} signal at the concentrations used in our assay.

Finally, RMI reduces the Ca^{2+}-dependent activation of adenylate cyclase (table 2), and S-100b counteracts the effect of this inhibitor (table 2). Results very similar to those reported here are obtained with purified sarcolemma membrane preparations (not shown).

4. DISCUSSION

The data presented in this report show that S-100b can modulate the activity of adenylate cyclase by markedly decreasing the activating action of Mg^{2+} and by increasing the stimulating effect of Ca^{2+}.

As to the S-100b effect in the presence of Ca^{2+}, it is noteworthy that the protein is equally effective at 0.1 mM Ca^{2+} as at 1 mM Ca^{2+}. The affinity of S-100 proteins for Ca^{2+} in the absence of monovalent cations is 0.05 mM [23]. Thus the ability of S-100b to regulate muscle adenylate cyclase may be achieved via conformational changes induced on it by Ca^{2+} at 0.1–1.0 mM [24].

Recent observations indicate that the affinity of S-100b protein for Ca^{2+} depends on its conformation and increases once this protein has interacted with its targets [25,26]. The findings registered in the presence of Mg^{2+} or a combination of this cation with RMI or NaF deserve some comments. The effect of S-100b in the presence of Mg^{2+} cannot be attributed to chelation of Mg^{2+} since this cation is present in large excess over S-100b and since this protein does not appreciably bind Mg^{2+} [27].

An attractive possibility is that S-100b counteracts the activating effect of Mg^{2+} by a direct interaction with some component(s) of the enzyme system. This conclusion is further strengthened by the finding that S-100b abolishes the activating effect of NaF which has been shown to affect the intermediate fraction of adenylate cyclase [21]. This hypothesis could be confirmed by experimental tests with the use of labeled S-100b and identification of protein components of the adenylate cyclase system with which S-100b interacts. Future studies should allow one to dissect the molecular mechanism involved in the opposite effects exerted by S-100b on adenylate cyclase depending on the metal present and, most important, whether the protein is also effective on this enzymatic system in an intact cell.

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