ACTIVATION OF SPLEEN ADENYLATE CYCLASE BY AGENTS FORMING MIXED DISULFIDES

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1. Introduction

In spite of extensive studies there is still little known about the molecular structure of the membrane-bound adenylate cyclase complex [1,2]. Nevertheless, there are some indications on the involvement of sulfhydryl and disulfide groups in the enzyme activity. It has been shown that several thiol group reagents inhibit adenylate cyclase [3–8]. Furthermore, 50% loss of adenylate cyclase activity with mercaptoethanol points out to the role of disulfide groups in the enzyme complex [9]. Similar results about the importance of vicinal dithiol groups for guanylate cyclase have been reported [10].

In contrast to the above inhibition of adenylate cyclase by agents affecting either –SH or S–S groups, the activation of spleen adenylate cyclase by mercaptoethanolamine and its respective disulfide (cystamine) has been demonstrated here.

2. Materials and methods

[U-^{14}C]ATP, spec. act. 573 mCi/mmol, was obtained from Radiochemical Centre, Amersham. ATP and adenosine 3′,5′-cyclic monophosphate [cAMP] were purchased from Calbiochem. Phosphoenolopyruvate, pyruvate kinase, 2-mercaptoethylamine–HCl, di-(aminoethyl)disulfide (cystamine) and bovine serum albumin Cohn fraction V were obtained from Koch-Light. Polyethyleneimine was purchased from British Drug Houses. Cellulose MN 300 was obtained from Machery-Nagel. PEI-cellulose was prepared by the method in [11]. PPO and POPOP were from Fluka.

CFW male mice, 3 months, were killed by cervical dislocation. The spleens were immediately removed, weighed and washed with 10 mM ice-cold Tris–HCl buffer (pH 7.5). Homogenization was carried out in 5 vol. same buffer in an all-glass homogenizer and the homogenate was strained through 4 layers of gause.

Mercaptoethanolamine and cystamine dissolved in 10 mM Tris–HCl (pH 7.5) were incubated with the homogenate (8–12 mg/ml) in a shaking water bath for 10 min at 37°C. Then 50 μl portions from each sample were taken to the adenylate cyclase reaction mixture.

Adenylate cyclase activity was determined following the conversion of [^{14}C]ATP to cyclic [^{14}C]AMP as detailed in [12]. The assay media contained 0.4 mM [U-^{14}C]ATP (16 μCi/μmol), 1 mM cAMP, an ATP-regenerating system (20 mM phosphenolpyruvate, 20 μg pyruvate kinase, 5 mM KCl), 40 mM Tris–HCl buffer (pH 8.2) and 0.3–0.6 mg of spleen homogenate protein in final vol. 150 μl.

The reaction, initiated by the addition of the homogenate, was run at 37°C for 10 min, and was stopped by 3 min boiling. The cAMP formed was isolated by thin-layer chromatography on PEI-cellulose with ethanol/0.5 M ammonium acetate (5:2) as the developing solvent. The radioactivity of cAMP was counted in Bray's solution [13] using a Nuclear Chicago Mark I liquid scintillation radiometer.

Protein was determined by the Lowry method [14].

3. Results and discussion

The effects of mercaptoethanolamine and cystamine in vitro on adenylate cyclase activity of the mouse

Table 1
Activation of adenylate cyclase of the mouse spleen homogenate by mercaptoethanolamine and cystamine

Addition	mM	Spec. act. (pmol cAMP/mg prot./min)
None		18.2 ± 1.4
Mercaptoethanolamine	0.1	41.4 ± 6.1
	1.0	46.2 ± 4.2
	10.0	37.5 ± 2.2
Cystamine	0.1	38.8 ± 3.1
	1.0	38.4 ± 4.9
	10.0	30.3 ± 2.2

Spleen homogenate was preincubated at 37° for 10 min in the presence of mercaptoethanolamine and cystamine as shown. Adenylate cyclase activity was measured as in section 2. Results are expressed as mean ± SD of four determinations.

spleen homogenate are shown in table 1. Both mercaptoethanolamine and cystamine were found to increase the enzyme activity by 2-fold. The activation occurred at the homogenate protein > 7 mg/ml. It might indicate that the activating effect of mercaptoethanolamine and cystamine depended on its ratio to the pool of reactive cysteine protein residues during the treatment.

It is well known that mercaptoethanolamine forms mixed disulfides by reacting with protein disulfide groups. Cystamine, on the other hand, reacts with disulfide as well as with thiol groups. The same influence of both agents on the adenylate activity strongly suggests their direct interaction with disulfide groups of the enzyme complex leading to the observed enzyme activation. It is worth emphasizing that while spleen adenylate cyclase activity was enhanced by cystamine brain adenylate cyclase was inhibited by DTNB (dithiobisnitrobenzoic acid), another disulfide compound but not forming mixed disulfides [5].

At present it is difficult to answer whether the noticed effects were due to direct interaction of mercaptoethanolamine and cystamine with the enzyme itself or with any regulatory protein of the enzyme complex. Nevertheless, the results seem to indicate the role of S–S bridges in adenylate cyclase complex. Thus, the activation of spleen adenylate cyclase following the i.p. administration of mercaptoethanolamine and cystamine, described in [12,15], may also be considered to result from formation of mixed disulfides with critical S–S groups of the enzyme complex.

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