Rp-diastereomer of adenosine cyclic 3′,5′-phosphorothioate as an antagonist of the stimulatory action of cyclic AMP on the ouabain-insensitive Na efflux in single barnacle muscle fibers [1]

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Summary. Single muscle fibers of the barnacle *Balanus nubilus* have been used as a preparation to test the possibility that the Rp-diastereomer of adenosine cyclic 3′,5′-phosphorothioate, which is the first available analog of cAMP that acts as an antagonist of cAMP, may reduce the magnitude of cAMP-mediated stimulation of the resting ouabain-insensitive Na efflux. The results obtained show that this antagonist is, in fact, able to reduce stimulation of the Na efflux by injected cAMP in a dose-dependent manner.
Key words. cAMP; analog; antagonist; sodium efflux; barnacle muscle fibers.

Earlier work with single barnacle muscle fibers has shown that injection of cAMP causes stimulation of the ouabain-insensitive Na efflux [3], a response that is reduced by the preinjection of protein kinase inhibitor [4] (PKI) or regulatory subunit of cAMP-protein kinase [5]. It is also reduced by the preinjection of synthetic PKI [6]. The recent studies by Rothermel and coworkers [7–9] of an Rp-diastereomer of adenosine cyclic 3′,5′-phosphorothioate (Rp-cAMPS) that prevents the dissociation of cAMP-protein kinase by cAMP has prompted us to test the hypothesis that it may also interrupt the stimulatory action of injected cAMP on the ouabain-insensitive Na efflux. The purpose of this communication is to provide evidence that this cAMP analog is a genuine but partial antagonist of cAMP when it is microinjected into the barnacle muscle fiber preparation prior to cAMP.

Material and methods. Specimens of *Balanus nubilus* were supplied by the Pacific Biomarine Laboratory Inc., Inglewood, CA. They were kept in a filtered, aerated, aquarium containing artificial seawater. The temperature of the aquarium water was maintained at ~12 °C. Single fibers measuring 3–4 cm in length and 1–2 mm in width were isolated by dissection from the depressor muscle bundles and then cannulated in the same way as *Maia* muscle fibers [10]. The artifical seawater (ASW) used in these experiments had the following composition (mM): NaCl, 465; KCl, 10; $MgCl_2$, 10; $CaCl_2$, 10; $NaHCO_3$, 10; and pH 7.8. Test solutions for injection were prepared using a 3 mM-HEPES solution, pH 7.2. The microinjector used was of the type described by Bittar and Tallitsch [11]. The volume of test fluid injected into the fibers was about 0.4 µl. Since such fibers have an intrafiber fluid volume of approximately 40 µl, dilution by the myoplasm may be taken as 100-fold. ^{22}NaCl was obtained from Amersham-Searle Corporation. The effluent samples from the cannulated fibers loaded with radiosodium were collected at 5-min intervals. The activities of these samples and that of the fiber at the end of the experiment were determined by counting in a Beckman gamma counter. The data were processed by an Apple II computer programmed to calculate both ^{22}Na efflux (cpm per min) and the fractional rate constant for ^{22}Na loss. Changes in Na efflux were estimated on the basis of the rate constant plots and expressed as a percentage change (mean ± SE of mean) of the efflux. Student's t-test was used to compute significance levels. All experiments were done at room temperature, 23 ± 1 °C. Ouabain, HEPES and cyclic AMP were purchased from Sigma Chemcial Company, St. Louis, MO. Rp-diastereomer of adenosine cyclic 3′,5′-phosphorothioate (Rp-cAMPS) was a gift from Dr Jeffrey Nadelson of the Sandoz Research Institute, Sandoz, Inc., East Hanover, N.J.

Results and discussion. In the first group of experiments, fibers pretreated with 10^{-4}M-ouabain were injected with 10^{-3}M-Rp-cAMPS (in a 3 mM-HEPES solution, pH 7.2), followed 30 min later by an injection of 10^{-3}M-cAMP. Companion controls were injected with a 3 mM-HEPES solution prior to 10^{-3}M-cAMP. At first sight, this particular concentration of cAMP may seem rather high, but not if one takes into account such factors as: a) 100-fold dilution by the myoplasm; b) loss of a fraction of the injected cAMP as an *efflux*, and c) the presence of a brisk cAMP-PDE system in these fibers. A concentration of 10^{-5}M (after dilution) is reasonable, particularly since it is about 50 times the average concentration of cAMP in barnacle fibers [4]. The results obtained in these experiments show the magnitude of the stimulatory response in the test fibers as averaging 161 ± 8%, n = 6 vs 419 ± 63%, n = 6 in the controls. The difference is significant, p being <0.01. A representative test and control experiment is shown in figure 1 a and b, where it can be seen that the kinetics of the stimulatory response are alike in both situations; that is, the response to cAMP is prompt in onset and transitory but its size in the test fiber is considerably smaller than that observed in the companion control. Such a result is in line with the observation that Rp-cAMPS acts by competing with cAMP for binding to the cAMP-protein kinase regulatory subunits [8, 12].

These results led to the construction of a concentration-response curve, based on the injection of 10^{-3}M-cAMP into ouabain-poisoned fibers preinjected with Rp-cAMPS in

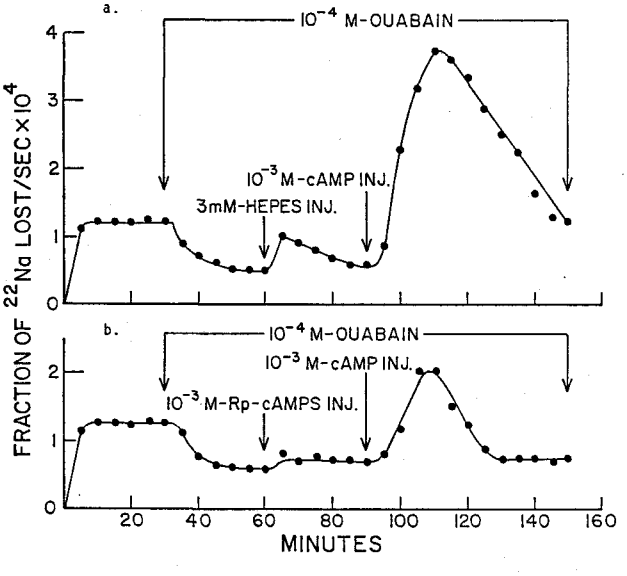

Figure 1. *a* Effect on the ouabain-insensitive Na efflux of injecting a 3 mM-HEPES solution, followed by a 10^{-3}M-cAMP solution (rate constant for ^{22}Na efflux plot). *b* Effect on the ouabain-insensitive Na efflux of injecting 10^{-3}M-Rp-cAMPS, followed by 10^{-3}M-cAMP. Microinjection of a substance involves its dilution by the myoplasm by a factor of roughly 100.

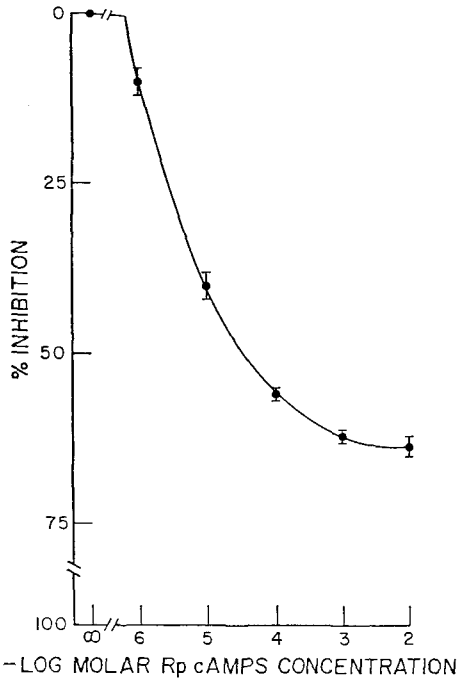

Figure 2. Dose-response curve for the inhibitory action of Rp-cAMPS on the response of the ouabain-insensitive Na efflux to the injection of 10^{-3}M-cAMP. Each plotted point represents the mean value of 3 determinations. Vertical bars indicate ±SE of mean. The fibers were isolated from the same barnacle specimen.

varying concentration. As shown in figure 2, preinjection of 10^{-6}M-Rp-cAMPS exerts a rather minimal inhibitory effect (viz 10%), whereas a 10^{-3}M- or 10^{-2}M-solution exerts a maximal inhibitory effect on the response of the ouabain-insensitive Na efflux to the injection of 10^{-3}M-cAMP. The magnitude of the latter effect is about 61%. Thus, the IC_{50} falls in the region of 5×10^{-6}M (using a log scale). The fact that Rp-cAMPS does not completely abolish the response of the Na efflux to cAMP when it is injected in an equimolar concentration cannot be readily attributed to the breakdown of the analog by cAMP phosphodiesterases (low and high K_m forms), since it is known to be resistant to hydrolysis[13,14]. However, there is an exception[8], namely the case of yeast cAMP-PDE, the apparent K_m for Rp-cAMPS being 1×10^{-4}M. If this is also true of barnacle muscle cAMP-PDE, one would then expect Rp-cAMPS to interfere with the breakdown of injected cAMP, thus resulting in a greater response of the Na efflux to cAMP. Another but simpler explanation is that Rp-cAMPS behaves as a partial antagonist in situ of the cAMP-induced activation of cAMP-protein kinase. Furthermore, it must not be overlooked that in experiments of this type the injection of a substance axially and uniformly along the entire length of the fiber cannot be claimed with certainty.

Another means of studying the efficacy of Rp-cAMPS is to determine whether it reduces the magnitude of the stimulatory response of the Na efflux to high external K^+. Experiments therefore were done in which test and control fibers were suspended suddenly in 100 mM-K^+-ASW after injection of 10^{-3}M-Rp-cAMPS and 3 mM-HEPES into them, respectively. The results obtained are as follows: 131 ± 17% stimulation, n = 5, vs 220 ± 22%, n = 5 in controls. The difference is significant ($p < 0.02$). This finding confirms an earlier conclusion that the response of the Na efflux to high external K^+ or injection of Ca^{2+} is mediated in part by the activation of cAMP-protein kinase by newly formed cAMP[15,16].

In conclusion, these observations provide evidence that Rp-cAMPS is a partial antagonist of cAMP and that it promises to be a powerful tool at the disposal of the cell physiologist. Whether it also acts as a partial antagonist in barnacle fibers when the internal cAMP level is raised as the result of activation of the membrane adenylate cyclase system in situ by specific agonists remains to be seen.

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Studies on correlations between chloroquine-induced tissue damage and serum enzyme changes in the rat[1]

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Summary. The administration of chloroquine to rats resulted in a significant elevation of serum enzymes and a corresponding decrease of these enzymes in the tissues. The changes in serum and kidney enzymes were most marked, thus indicating a primary renal dysfunction.
Key words. Rats; chloroquine; renal damage; lysosomes; acid hydrolases; membrane damage.