ACTIVATION OF TURKEY ERYTHROCYTE ADENYLATE CYCLASE BY TWO RECEPTORS: ADENOSINE AND CATECHOLAMINES

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1. Introduction

Adenosine has been known for some time to activate adenylate cyclase through its interaction with adenosine receptors [1–8]. In experiments conducted in our laboratory we have occasionally observed that if one eliminates theophylline from the adenylate cyclase assay of turkey erythrocyte membranes significant basal activities are observed. Whereas in the presence of 1.0–2.0 mM theophylline or adenosine antagonists [3], the turkey erythrocyte adenylate cyclase exhibits a basal activity of less than 1.0 pmol cAMP/min/mg, in the absence of theophylline values of up to 100 pmol/min/mg could be observed. In the presence of adenosine deaminase the basal activity of these preparations could be diminished to values similar to those observed in the presence of theophylline. Due to the fact that not every membrane preparation could be stimulated by adenosine we sought a more definite experiment which would determine whether an adenosine receptor exists in these cells. We indeed found that in every membrane preparation of turkey erythrocytes the adenylate cyclase could be activated to its permanently active form [9–10] by adenosine and GppNHp. The ability of adenosine and GppNHp to activate the adenylate cyclase in a synergistic fashion was found to be very similar to the process of enzyme activation by l-epinephrine and GppNHp [9–10]. As reported previously [9–10] GppNHp alone cannot induce enzyme activation. The preliminary characterization of this process is the subject of this communication.

Abbreviations: GppNHp, guanylylimidodiphosphate

2. Materials and methods

[α-^{32}P]ATP was obtained from Radiochemical Centre (Amersham, England). All chemicals used were of the highest degree of purity available. All solutions were prepared in Corning doubly distilled water. Adenylate cyclase was assayed according to Salomon et al. [11] and protein was determined according to Lowry et al. [12]. Turkey erythrocyte membranes were prepared as previously described [13]. Other experimental details are given in the legends to the figures.

3. Results

3.1. *The activation of adenylate cyclase by adenosine and GppNHp*

When turkey erythrocyte membranes are incubated with increasing concentrations of adenosine, in the presence of saturating GppNHp, the adenylate cyclase is activated to its permanently active form (fig.1). The process of activation can be stopped at any time by the addition of theophylline, an adenosine antagonist [3]. Theophylline, however, is unable to revert the permanently active enzyme back to its inactive form. Since DL-propranolol was present during all incubations the possibility that the adenosine effect is through the β-adrenergic receptors can be ruled out. The maximal level of activation attainable by adenosine plus GppNHp is 70% of the maximal level attainable with l-epinephrine and GppNHp. Other nucleosides such as guanosine were found to be without any effect on the adenylate cyclase system.

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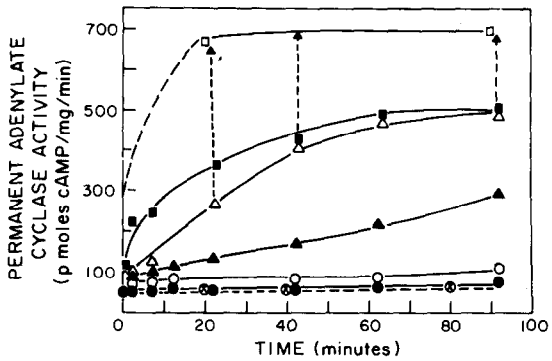

Fig.1. Kinetics of adenylate cyclase activation by adenosine and GppNHp. Turkey erythrocyte membranes at a final concentration of 0.72 mg/ml were preincubated with 1.0×10^{-5} M GppNHp in 50 mM Tris–HCl buffer, pH 7.4, containing 2 mM $MgCl_2$ and 1 mM EDTA, at 37°C. At zero time adenosine in the same buffer at a final concentration indicated in the figure was added, also after prior incubation at 37°C. At time points indicated by the experimental points, theophylline at a final concentration of 1.1 mg/ml was added, to stop the activation process. Adenylate cyclase activity was then measured in these samples in the presence of 0.58 mg/ml theophylline final concentration. The assay also contained 1.0×10^{-5} M DL-propranolol. The dashed arrows indicate that the non-activated enzyme, remaining after theophylline addition can be activated by the addition of l-epinephrine, to its maximal attainable specific activity. (-●-●-) Control, containing 2.2 mM theophylline plus 1.0×10^{-5} DL-propranolol and 1.0×10^{-5} M GppNHp. (-⊗-⊗-) Control containing 2.2 mM theophylline plus 1.0×10^{-5} M GppNHp. (-○-○-) 1.0×10^{-7} M adenosine. (-▲-▲-) 1.0×10^{-6} M adenosine. (-△-△-) 1.0×10^{-5} M adenosine. (-■-■-) 1.0×10^{-4} M adenosine. (-□-□-) 1.0×10^{-5} M l-epinephrine.

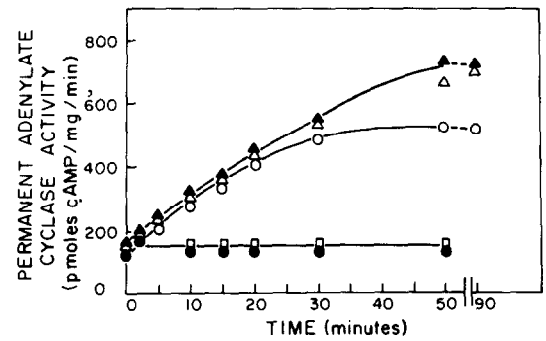

Fig.2. The combined activation of adenylate cyclase by adenosine and l-epinephrine, in the presence of GppNHp. The experimental details are similar to those given in the legend to fig.1. All reaction mixtures contained 1.0×10^{-5} M GppNHp final concentration. The adenosine and l-epinephrine concentrations used in this experiment were 1.0×10^{-6} M and 1.0×10^{-7} M, respectively. When adenosine was used alone the process of enzyme activation was stopped by theophylline and when the enzyme activation was induced by l-epinephrine alone the reaction was stopped with 1.0×10^{-5} M DL-propranolol. When the enzyme activation was induced by both adenosine and l-epinephrine the reaction was stopped by a mixture of 1.0×10^{-5} M DL-propranolol and 1.1 mg/ml theophylline final concentration. (-○-○-) 1.0×10^{-6} M adenosine. (-△-△-) 1.0×10^{-7} M l-epinephrine. (-▲-▲-) 1.0×10^{-6} M adenosine and 1.0×10^{-7} M l-epinephrine. (-□-□-) 1.0×10^{-5} M GppNHp. (-●-●-) 1.0×10^{-5} M GppNHp plus 1.0×10^{-5} M propranolol and 1.1 mg/ml theophylline.

3.2. *The rate of enzyme activation by adenosine*

The rate of adenylate cyclase activation by adenosine, in the presence of GppNHp, is maximal at 1.0×10^{-4} M and half-maximal between 1.0×10^{-6} M and 1.0×10^{-5} M (fig.1). This indicates that the affinity of adenosine to its receptor is between 1 μM and 10 μM. The maximal rate of activation by adenosine is much slower than the maximal rate of activation induced by l-epinephrine and GppNHp.

3.3. *The combined action of adenosine and l-epinephrine*

l-Epinephrine at 1.0×10^{-7} M and adenosine at 1.0×10^{-6} M activate adenylate cyclase at comparable rates (fig.2), in the presence of GppNHp. It can be seen that when the two ligands are combined the rate of enzyme activation is less than the sum of the rates of activation induced by the two ligands separately (fig.2). This finding indicates that the two receptors compete for a common pool of enzyme and their effect is not additive. This non-additivity of the adenosine and epinephrine action could be demonstrated over a wide concentration of the two ligands. The concentrations used in the experiment described in fig.2 are in the most sensitive range for the detection of additivity.

4. Discussion

4.1. *The pattern of adenosine activation*

Turkey erythrocyte membranes possess both a β-adrenergic receptor and an adenosine receptor. The

occupancy of these receptors by either adenosine or 1-epinephrine induce the activation of the enzyme adenylate cyclase. Adenosine in the presence of GppNHp induces the activation of adenylate cyclase to a permanently active state by amichanism similar to the process of adenylate cyclase activation by 1-epinephrine and GppNHp [9–10]. Theophylline, an adenosine antagonist, inhibits the process of enzyme activation by adenosine and GppNHp but is unable to revert the permanently active enzyme back to its inactive form. This pattern of behavior of the adenosine antagonist is similar to the behavior of propranolol with respect to the process of enzyme activation by 1-epinephrine and GppNHp [9–10].

4.2. *The non-additivity of adenosine and epinephrine action*

Adenosine is capable to activate 70% of the enzyme pool to its permanently active state, in the presence of GppNHp (fig.1). All of the enzyme pool, however, can be activated to its permanently active state by 1-epinephrine and GppNHp (fig.1). Thus the maximal activity attainable in the presence of both 1-epinephrine and adenosine and in the presence of GppNHp is identical to the maximal activity attainable with 1-epinephrine alone, in the presence of GppNHp (fig.1 and 2). When both adenosine and 1-epinephrine are present together, the rate of enzyme activation in the presence of GppNHp, is less than the sum of rates of the two ligands, when present separately (fig.2). The non-additivity of the adenosine and the 1-epinephrine effects is observed over a wide concentration range of adenosine and 1-epinephrine. These results indicate that the two receptors compete for a common pool of adenylate cyclase. It seems that the turkey erythrocyte system can become a suitable model system to study the mode of interaction of one adenylate cyclase with two different receptors.

5. Conclusions

Adenosine in the presence of guanylylimidodiphosphate (GppNHp) was found to induce the formation of a permanently active adenylate cyclase. This effect of adenosine is inhibited by the adenosine antagonist theophylline but the latter is unable to revert the enzyme to its inactive state. 70% of the total adenylate cyclase pool was found to be sensitive to adenosine whereas the remaining 30% could be activated only by 1-epinephrine and GppNHp. All of the enzyme pool could be activated by 1-epinephrine and GppNHp. The combined action of adenosine and 1-epinephrine indicates that their action is not additive and both compete for a common pool of adenylate cyclase.

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