# SLOW GDP DISSOCIATION FROM THE GUANYL NUCLEOTIDE SITE OF TURKEY ERYTHROCYTE MEMBRANES IS NOT THE RATE LIMITING STEP IN THE ACTIVATION OF ADENYLATE CYLASE BY β-ADRENERGIC RECEPTORS

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## 1. Introduction

It is well established (reviewed [1]) that the activation of β-receptor-dependent adenylate cyclase requires the simultaneous occupancy of the β-receptor by agonist and of the GTP regulatory site by GTP. The hormonal signal is terminated concomitantly with the hydrolysis of GTP to GDP and $P_i$ [2], most probably at the regulatory site. The reactivation of the system therefore requires the dissociation of GDP [3–6] from the regulatory site and the binding of a new molecule of GTP. Either or both of the latter steps require the presence of an agonist; namely, a fruitful interaction between the agonist-bound receptor and the cyclase system is required. In [7] it was argued that the rate limiting step in the activation of adenylate cyclase is the dissociation of GDP from the regulatory GTP site. Here I argue, on the basis of the data in [7], that the dissociation of GDP from the regulatory site of the turkey erythrocyte adenylate cyclase is not the rate limiting step in the hormone-dependent adenylate cyclase activation.

## 2. Results and discussion

In [7], 1 figure and 2 tables are presented which depict time courses of cAMP formation in the presence of saturating hormone concentrations and saturating Gpp(NH)p concentrations. Such kinetic data should yield two fundamental parameters:

(i) The maximal specific activity ($V_{max}$) which is represented by the limiting slope of the time course of cAMP formation;

(ii) The rate constant ($k_{on}$) which characterizes the rate of adenylate cyclase activation by the hormone-bound receptor [5,8–10].

The fundamental equation [8–10] which describes this first order in process is:

$$[\mathrm{cAMP}]_t = V_{max} \cdot t + \frac{V_{max}}{k_{on}} \{\exp(-k_{on})t - 1\} \quad (1)$$

We have used this phenomenological equation to analyze the data in [7].

### 2.1. *Analysis of data*

On the basis of the numbers presented in table 1 of [7], one can compare graphically (fig.1) the kinetic pattern of cyclase activation of 'GDP-depleted membranes' and 'GDP-loaded membranes'. It is immediately apparent that the 'GDP-loaded membranes' and the 'GDP-depleted membranes' exhibit an identical lag time (~5 min). The latter is a measure of $k_{on}$ (see [8–11]). Indeed, if one fits the data to eq. (1) as described [8–11], one obtains $k_{on} = 0.2 \pm 0.045$ min$^{-1}$ for both systems. $V_{max}$ is, however, higher for the 'GDP-depleted membranes'. This is most probably due to the loss of protein from the EDTA-treated membranes. EDTA was used to deplete the membranes from GDP which we have found to remove 15–25% of the proteins from turkey erythrocyte membranes (A. L., unpublished). In summary, the data in [7] demonstrate that within experimental error the removal of GDP from the regulatory site does not

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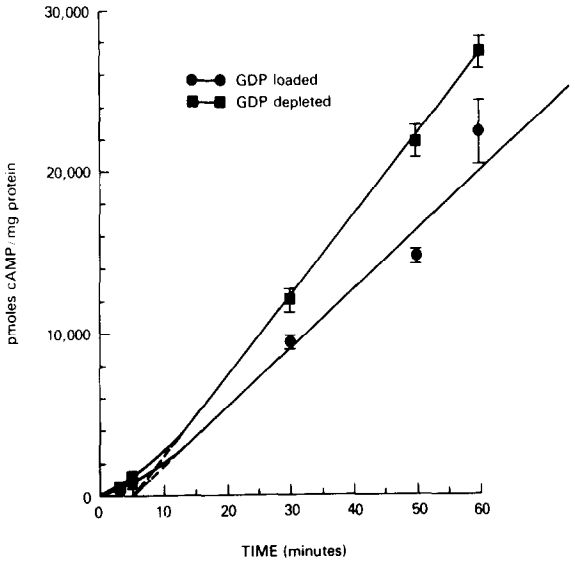


Fig.1. cAMP accumulation due to stimulation by isoproterenol and Gpp(NH)p of 'GDP-loaded membranes' and 'GDP-depleted membranes'. The experimental data are taken from [7].

modify the kinetics of cyclase activation by hormone and Gpp(NH)p.

### 2.2. *The collision coupling model*

It is stated in the discussion of [7] that the collision coupling mechanism 'has been refuted on the basis of theoretical considerations', implying that the findings in [7] are an experimental corroboration. We would like to point out that the collision coupling mechanism [8,9] does not specify the chemical step which is involved upon the encounter between the agonist bound receptor and the cyclase system. The chemical step can involve the removal of GDP from the regulatory site, as indeed is proposed in [3–6]. Whether the mechanism of cyclase activation is collision coupling or not, the chemical steps involved are obviously identical, as pointed out in [8–10, 12–14] but of which the authors of [7] may be unaware.

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