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# Kinetics of interaction between β-receptors, GTP protein, and the catalytic unit of turkey erythrocyte adenylate cyclase

(collision coupling)

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**ABSTRACT** **The kinetics of turkey erythrocyte membrane adenylate cyclase activation by β-agonists and guanyl-5′-yl imidodiphosphate is explored as a function of the concentration of the GTP regulatory protein and of the catalytic unit. It was found that the overall kinetics of activation is first order and is independent of the concentration of the GTP regulatory unit N, the catalytic unit C, and of hormone over a very wide concentration range. It was established that the rate-limiting step does not involve GDP dissociation from the inactive N unit or the association between activated N′ and C. Also, it was found that guanyl-5′-yl imidodiphosphate binding occurs in a random fashion and is not hormone dependent. These results enable us to exclude models of the sequential type in which N in its inactive form is bound to receptor R, is released in an active form N′ upon hormone activation, and then binds to C, activating the latter. An acceptable model that accounts for all of the data conforms to the original formulation of "collision coupling" in which N is tightly associated to C at all times.**

In turkey erythrocytes the activation of the inactive form of the enzyme C to its active form C′ is initiated by the occupancy of the receptor R by the β-adrenergic hormone H and of the guanyl nucleotide regulatory protein N by certain guanyl nucleotides (1–3).

C′ accumulates irreversibly in the presence of hormone and guanyl-5′-yl-imidodiphosphate (p[NH]ppG) (1, 2) or guanosine (5′→$O^3$)-1-thiotriphosphate (GTP[γS]) (4). The time course of C′ accumulation can be followed, which enables one to test the role of the hormone H and p[NH]ppG and the role of the components R, N, and C in determining the sequence of events leading to C′ accumulation. The first set of such experiments described the appearance of C′ as a function of H and R concentrations in the presence of saturating p[NH]ppG concentrations (5). The major finding that was derived from these experiments was that the kinetics of C′ accumulation was found to be first order. The apparent rate constant of activation of C, $k_{on}$, depended linearly on the total receptor concentration $[R_T]$ and on the saturation function for the hormone H:

$$k_{on} = k[R_T][H]/(K_H + [H]) \quad [1]$$

The half saturation constant, $K_H$, is identical with the hormone–receptor dissociation constant obtained from binding experiments. This equation contains the proportionality constant, $k$, which may include still additional parameters. Indeed, we have not addressed ourselves in our analysis (5) to the kinetic role of the GTP regulatory unit N and of the nucleoside triphosphate p[NH]ppG. It has been suggested that during the process of H activation, N is initially bound to R and is activated to N′ upon agonist occupancy. Subsequently N′ dissociates from HR and associates with the catalytic unit C to form the activated enzyme N′·C (6, 7).

In order to resolve the roles of N and of p[NH]ppG, both must be defined in models that describe the activation process. In this communication we attempt to continue the dissection of $k_{on}$ (Eq. 1) by examining the kinetic features of the interaction between R, N, and C in the presence of different concentrations of p[NH]ppG, at various constant concentrations of H, N, and C.

## MATERIALS AND METHODS

Turkey erythrocyte membranes were prepared as described (8). Cyclic AMP (cAMP) was determined as described (9); the assay contained about 70 μg of membranes, 4 mM $MgCl_2$, 1 mM disodium ATP, 10 units of creatine kinase, 20 mM disodium creatine phosphate, 50 mM Tris·HCl (pH 7.4), 2.2 mM theophylline, and 1–2 μCi (1 Ci = 3.7 $10^{10}$ becquerels) of [α-$^{32}$P]ATP, which was purchased from the Radiochemical Center (Amersham, England). Other experimental details are in the figure legends. Protein was assayed as described (10) with bovine serum albumin as a standard.

## RESULTS

**Activation of Adenylate Cyclase in the Presence of Limiting p[NH]ppG Concentrations.** Fig. 1 shows the time course of C activation at increasing p[NH]ppG concentrations. No change in the overall first-order kinetics of activation and in the magnitude of the first-order rate constant occurred, although the extent of enzyme activation diminished progressively with decreasing p[NH]ppG concentrations (Fig. 1*B*). Because the rate of activation was independent of p[NH]ppG concentration, it appears that the nucleotide binding is never rate limiting. We examined the possibility that the depletion of p[NH]ppG from N to nonspecific sites is the reason for the limited accumulation of C′ at low p[NH]ppG concentration. For this purpose we examined the effect of added untreated native membranes on the onset of cyclase activation in GMP/l-epinephrine-treated membranes.

In membranes pretreated with l-epinephrine/GMP and washed, 100 nM p[NH]ppG activates the enzyme, in the presence of propranolol, to its maximal extent rapidly and without a lag time (11). The addition of untreated membranes and 100 nM p[NH]ppG to treated membranes caused a rapid and irreversible depletion of p[NH]ppG to the 1 nM range (Fig. 2). Therefore, this rapid depletion of p[NH]ppG cannot account

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Abbreviations: p[NH]ppG, guanyl-5′-yl-imidodiphosphate; MalNEt, *N*-ethylmaleimide; cAMP, cyclic AMP.

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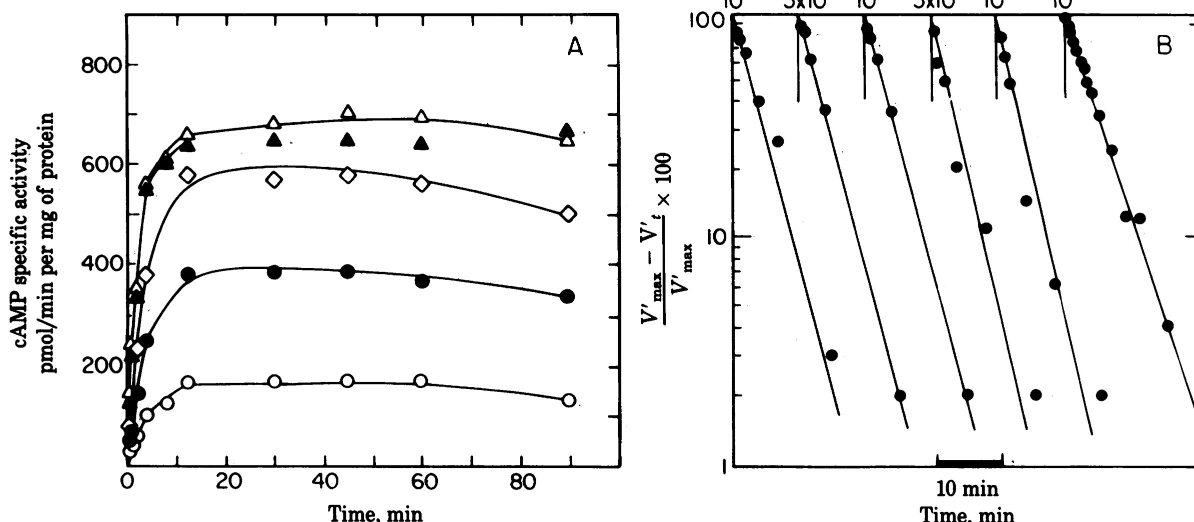



FIG. 1. The activation of adenylate cyclase as a function of p[NH]ppG concentration. (*A*) Turkey erythrocyte membranes (1.3 mg/ml) were incubated at 37°C with 0.1 mM 1-epinephrine and the following total p[NH]ppG concentrations: 0.01 μM, (○) 0.05 μM, (●) 0.1 μM, (◇) 0.5 μM, (▲) 1.0 μM, (△). At various times, as indicated by the data points, 70 μl was removed into 40 μl of ice-cold 10 μM *dl*-propranolol. After the entire time course was completed, 40 μl of assay cocktail was added, and cyclic AMP (cAMP) was accumulated for 20 min at 37°C. (*B*) The results presented in *A* and results similarly obtained at even higher p[NH]ppG concentrations (10 μM) are replotted here. $V_{max}$ is the maximal extent of activation obtained for each individual p[NH]ppG concentration. Thus, the fractional activation is *not* calculated to a common plateau. The concentration of p[NH]ppG is written at the top of each time course. $V'_t$, velocity at time *t*.

either for the slow activation of C or for the limited extent of C activation at limiting p[NH]ppG (Fig. 1). It seems that the extent of enzyme activation depends on the amount of free p[NH]ppG where the nucleotide becomes irreversibly bound during the course of activation. As a result, increasing the concentration of membranes at constant p[NH]ppG concentrations limited the extent of enzyme activation (Fig. 3). Thus, at low membrane concentrations, 0.05 μM p[NH]ppG was equipotent with 10 μM p[NH]ppG (Fig. 3*C*).

**Hormone Concentration Dependence at Increasing p[NH]ppG Concentrations.** Fig. 4 shows that the hormone concentration dependence of enzyme activation was independent of p[NH]ppG concentration. Regardless of the extent of enzyme activation, the process was first order, and the apparent dissociation constant for 1-epinephrine for this process was found to be $1.8 \pm 0.1 \times 10^{-6}$ M.

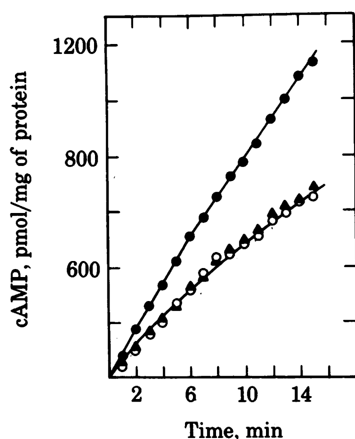



FIG. 2. Depletion of p[NH]ppG by native membranes. Membranes (1.3 mg/ml) were incubated for 10 min at 37°C with 0.1 mM GMP and 0.1 mM 1-epinephrine in 50 mM Tris·HCl, pH 7.4/2 mM $MgCl_2$/1 mM $Na_2EDTA$. Then the membranes were diluted 1:10 in the same ice-cold buffer and centrifuged (23,000 × *g*, 10 min). This washing was repeated three times. GMP-treated membranes were assayed in 1.0 nM (○) or 100 nM (●) p[NH]ppG at a final concentration of 0.44 mg/ml. Reaction was started by addition of the standard assay mixture containing p[NH]ppG and 1.0 μM *dl*-propranolol at 25°C. In parallel, "GMP-treated" membranes (▲) were mixed prior to assay with native membranes (0.52 mg/ml) and then assayed in the presence of 100 nM p[NH]ppG and 1.0 μM *l*-propranolol.

**Kinetics of Enzyme Activation in GDP-Depleted Membranes.** Because it was suggested that the release of GDP from *N* is the rate-limiting step of cyclase activation (12), we examined the kinetic properties of membranes that were treated to deplete them of tightly bound nucleotides. Two nucleotide-free preparations were used. One nucleotide-free preparation was obtained by EDTA treatment (13) and the second was obtained by GMP plus 1-epinephrine treatment, followed by extensive washing. EDTA-treated membranes were indistinguishable from native membranes in their kinetic properties (Fig. 5). GMP/1-epinephrine-treated membranes were activated by p[NH]ppG with almost no lag time (Fig. 5), as is also apparent from Fig. 2. However, upon incubating the nucleotide-free membranes at 30–37°C, the properties of the enzyme reverted to those of the native state (Fig. 5).

**Kinetics of C Activation After *N*-Ethylmaleimide (MalNEt) Treatment of Native Membranes.** MalNEt treatment of turkey erythrocyte membranes causes an irreversible loss in the ability of C to be activated by N. Even subsequent to 95% inactivation, the kinetic pattern of C activation by 1-epinephrine and p[NH]ppG remained identical to that of native membranes (Fig. 6). A reduction by a factor of 1000 in p[NH]ppG did not alter this pattern. In all cases the intrinsic rate constant of activation was $k_{on} = 0.8 \pm 0.2$ $min^{-1}$ at 37°C.

## DISCUSSION

**Overall Kinetic Pattern.** The kinetics of native turkey erythrocyte membrane adenylate cyclase activation by 1-epinephrine and p[NH]ppG were first order under all experimental conditions examined. This observation limits the number of molecular models for the interaction between the receptor R, the GTP regulatory unit N, and the catalytic unit C. Previous studies, which explored the kinetics of the system as a function of R con-

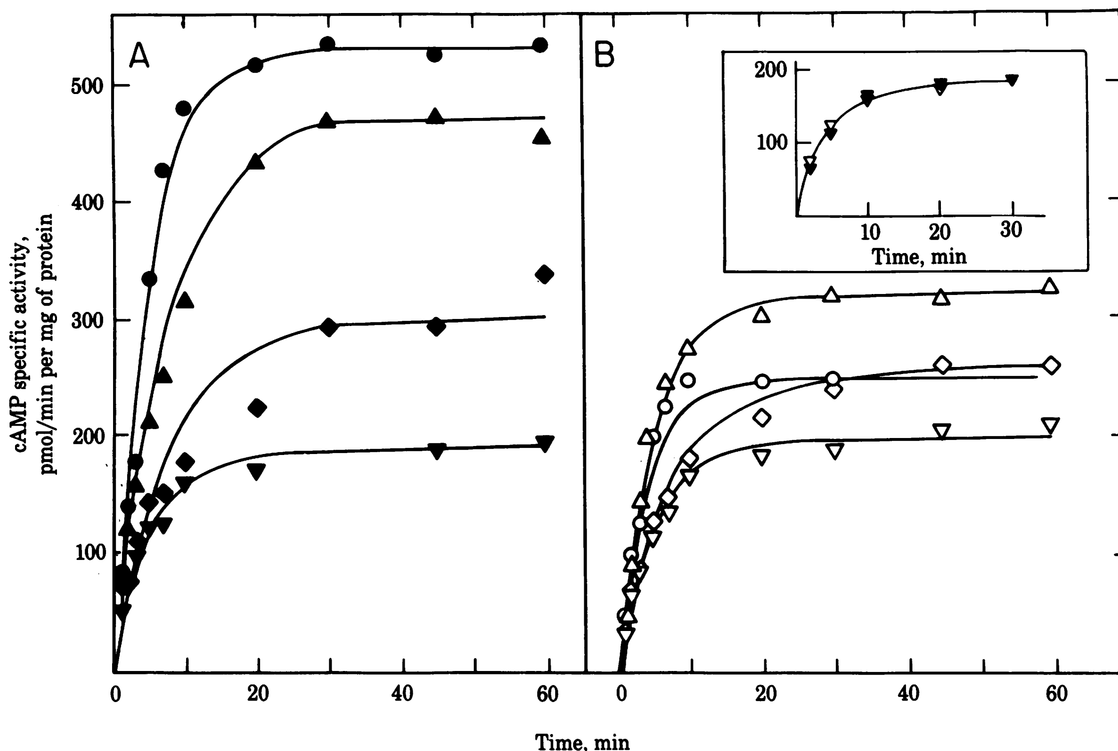



FIG. 3. The kinetic pattern of 1-epinephrine plus p[NH]ppG activation at different membrane concentrations. The membranes were preincubated with 10 $\mu$M 1-epinephrine and p[NH]ppG at 10 $\mu$M (*A*) and 0.05 $\mu$M (*B*) at 37°C in 2 mM $MgCl_2$/1 mM EDTA/50 mM Tris·HCl, pH 7.4, and the kinetics of activation of the enzyme were followed as described in Fig. 1. Membranes in preincubation: ○ and ●, 1 mg/ml; △ and ▲, 0.3 mg/ml; □ and ■, 0.1 mg/ml; ▽ and ▼, 0.05 mg/ml. The rate constants were calculated for each individual concentration of p[NH]ppG and for each enzyme concentration. For all eight cases, the average rate constant was $k_{on}$ = 0.183 $min^{-1}$ ± 0.012 SD. The plateau levels are in pmol/mg per min: ●, 531; ▲, 468; ■, 300; ▼, 188; ○, 250; △, 343; □, 260; ▽, 200. (*Inset*) Results obtained at 0.05 mg/ml of membranes per ml for both p[NH]ppG concentrations.

centration, have shown that the $\beta$-receptor acts catalytically (4). This finding, taken together with other observations, led us to formulate the "collision coupling" mechanism of adenylate cyclase. In this mechanism N is precoupled to C and remains coupled during the course of the C-to-C′ transition (Table 1, model A).

However, it has been suggested (6, 7) that the GTP binding

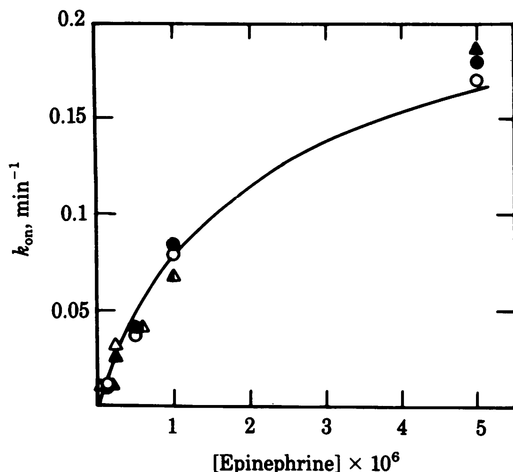



FIG. 4. The hormone dependence of the rate constant of adenylate cyclase activation at different p[NH]ppG concentrations. The apparent rate constant for activation of adenylate cyclase was determined by using an array of five different epinephrine and p[NH]ppG concentrations. p[NH]ppG concentrations: ○, 0.05 $\mu$M; ●, 0.10 $\mu$M; △, 0.25 $\mu$M; ▲, 0.5 $\mu$M. $K_H$ (Eq. 1) is 1.86 × $10^{-6}$ M for 1-epinephrine; the epinephrine concentration range examined was 0.1–50 $\mu$M. The line is theoretical, according to a $K_H$ value of 1.86 × $10^{-6}$ M and a $k_{on}$ = 0.226 $min^{-1}$.

protein N is bound to R and that, upon activation by H and GTP, the N unit migrates from R to C, activating the latter (Table 1, model B). Upon hydrolysis of GTP to GDP and the inactivation of $N'_{GTP}$ to $N_{GDP}$, the latter shuttles back to R. Decreasing the p[NH]pp concentration (Figs. 1, 2, 3, and 6) does not alter either the kinetic pattern of C activation or the absolute magnitude of the first-order activation constant ($k_{on}$). These findings suggest that the binding of p[NH]ppG to N is not rate limiting or else the magnitude of the rate constant of activation $k_{on}$ would depend on p[NH]ppG concentrations in a saturable manner. Thus, p[NH]ppG binds to N independently of H, and the H dependence of enzyme activation is independent of p[NH]ppG concentration (Fig. 4). Because the effect of p[NH]ppG is irreversible (Fig. 1; refs. 1 and 2), this nucleotide titrates and determines the number of units that can be activated by H·R and that can couple to C.

**GDP Release Is Not Rate Limiting.** The removal of GDP from native membranes does not alter the kinetics of cyclase activation by H and p[NH]ppG (Fig. 5). Nevertheless, one can alter the kinetics and shorten the lag time by GMP/1-epinephrine treatment. This is not a reflection of GDP depletion because such membranes can revert to slow kinetics, typical for native membranes, without involving guanyl nucleotides altogether (Fig. 5). Therefore, one must conclude that GDP release is not rate limiting, although its release may occur as soon as the rate-limiting step takes place.

**N-to-C Coupling.** The concentration of C was reduced by MalNEt treatment (Fig. 6). The reduction by a factor of 20 in the concentration of C that can couple to N had no effect on the kinetic pattern of activation. Furthermore, the combined concentration reduction of C to 1/20th and of N to 1/7th (Fig. 6) still had no effect on the kinetic pattern of activation. These

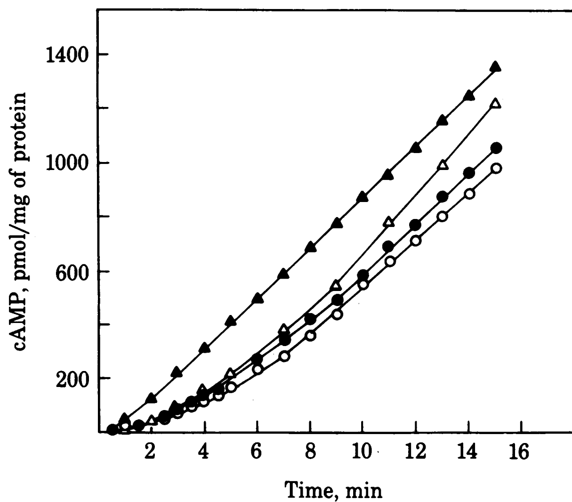



FIG. 5. Accumulation of cAMP in presence of epinephrine and Gpp(NH)p in native (●) and GDP-depleted membranes: GMP treated (▲), warm washed (○), and EDTA-treated native membranes (△). GMP-treated membranes were prepared as described in Fig. 2. Warm-washed membranes were treated with epinephrine and GMP and then centrifuged at 16,000 × g for 15 min at 30°C. Ten milliliters of 50 mM Tris·HCl, pH 7.4/2 mM $MgCl_2$/1 mM EDTA per mg of membrane protein was added at 36°C, and the membranes were incubated at this temperature for 5 min, followed by centrifugation. This operation was repeated three times. Final pellet was resuspended in cold buffer. Identical results are obtained upon incubation for 60 min at 25°C with no intermittent washings (data not shown). △, EDTA-treated membranes: membranes were treated at 37°C for 20 min with 5 mM EDTA/0.1 mM epinephrine/50 mM Tris·HCl, pH 7.4, and washed at 2–4°C.

observations show that the process of N-to-C coupling is not rate limiting.

**Molecular Models.** Any mechanism proposed for the mode of activation of turkey erythrocyte adenylate cyclase by hormones and p[NH]ppG must account for the following experimental observations. (*i*) the overall process is first order; (*ii*) the first-order rate constant is independent of the concentration of the catalytic unit and of the concentration of N; (*iii*) the first order rate constant of enzyme activation is directly proportional to $R_T$ (Eq. 1), but the extent of enzyme activation is independent of R concentration; (*iv*) the interaction of p[NH]ppG with N is not H dependent and determines the amount of N which can undergo activation; and (*v*) GDP dissociation is not rate limiting.

In Table 1 we examine all possible models in which N can either be preassociated with C prior to activation with the receptor R (model A), or can be activated by R prior to its interaction with C (model B). A third class is a hybrid model (model C) in which N interacts first with R and the activation occurs after the formation of a ternary complex R·N·C. Detailed mathematical derivations of these models, and the kinetic predictions which can be derived, are published elsewhere (14). The overall kinetics of activation and the contribution of C or of N, or both, to the rate law and to the magnitude of the rate constant $k_{on}$ were examined for each model. Models that do not conform to the following experimental results—(*i*) overall first-order kinetics of activation and (*ii*) no effect of reducing N or C, or both, on the rate law and on the rate constant $k_{on}$—must be rejected.

Three models conform to the experimental observations. (*i*) Model A assumes that N is always coupled to C. Indeed N can be resolved from C only in sodium cholate and high ionic strength (15). This would indicate that N′ and C are tightly coupled. Also, only slight N′-to-C dissociation has been reported for detergent-solubilized cyclase from brain (16). These studies would corroborate the notion that, even upon bilayer disruption, N′ and C are still tightly coupled. (*ii*) Model BIV, in which

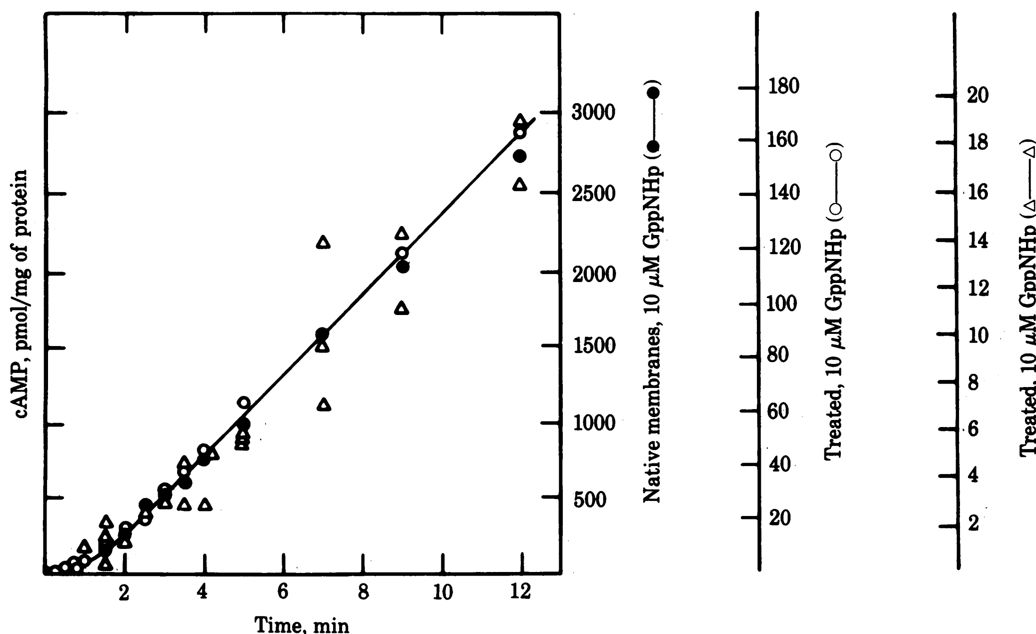



FIG. 6. The effect of MalNEt treatment on the kinetics of activation. Membranes were incubated at 0°C for 5 min with 5.0 mM MalNEt/50 mM Tris·HCl, pH 7.4/2.0 mM $MgCl_2$/1.0 mM $Na_2EDTA$, pH 7.4, followed by addition of 5.5 mM 2-mercaptoethanol. Membranes were washed four times in ice-cold buffer. MalNEt-treated membranes (○, △) and native membranes (●) were assayed at 37°C with 0.1 mM 1-epinephrine in the presence of 10 μM p[NH]ppG (○, ●) and of 0.01 μM p[NH]ppG (△). At the times indicated, aliquots were removed into 2% (wt/vol) $NaDodSO_4$/5.0 mM ATP/1.0 mM cAMP. Least squares nonlinear analysis of data (5) by the equation $[cAMP]_t = V_{max} \cdot t - V_{max}/k_{on}\{1 - \exp(-k_{on} \cdot t)\}$ produced the following values (with their SD) for $V_{max}$ (maximal specific activity in pmol/min per mg) and $k_{on}$ (in $min^{-1}$). Native membranes: $V_{max} = 265 \pm 0.4$, $k_{on} = 0.9 \pm 0.1$; MalNEt-treated membranes: 10 μM p[NH]ppG, $V_{max} = 15 \pm 5$, and $k_{on} = 0.8 \pm 0.2$; MalNEt-treated membranes: 0.01 μM p[NH]ppG, $V_{max} = 1.7 \pm 0.2$, and $k_{on} = 0.6 \pm 0.3$. $V_{max}$ of native membranes in the presence of 0.01 μM p[NH]ppG was found to be 37.1 pmol/min per mg. The line depicted is a theoretical one, with $k_{on} = 0.9\ min^{-1}$.

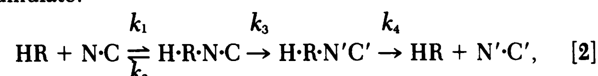
Table 1. Kinetic predictions of models of coupling between R, N, and C

| | Formulation | Rate equations for rate-limiting step | Overall kinetics |
|---|---|---|---|
| A. | $HR + NC \xrightarrow[a]{} (HRNC \rightleftharpoons HRN'C') \xrightarrow[b]{} HR + N'C'$ | $\frac{d[N'C']}{dt} = a[HR][NC]$ | First order |
| B. | $HR + N \xrightarrow[a]{} HRN \xrightarrow[b]{} HR + N' + C \xrightarrow[c]{} N'C'$ | | |
| BI. | R-to-N rate limiting | $\frac{d[N'C']}{dt} = a[HR][N]$ | First order |
| BII. | N-to-N′ conversion rate limiting | $\frac{d[N'C']}{dt} = b[HRN]$ | |
| | | (*i*) $[N_T] > [C_T] + [R_T]$ | Zero order |
| | | (*ii*) $[N_T] \simeq [R_T]$ | Non-first-order |
| | | (*iii*) $[N_T] > [R_T]$ | Non-first-order |
| | | (*iv*) $[N_T] \ll [R_T]$ | Non-first-order |
| BIII. | N′-to-C coupling rate limiting | $\frac{dN'C'}{dt} = c[N'][C]$ | Second order for C and N |
| BIV. | R-to-N conversion limits rate, rapid equilibrium between N′ and C | $\frac{dN'C'}{dt} = a[HR][N]$ | Almost first order except when $C_T \ll N_T$ |
| BV. | N-to-N′ conversion rate limiting, rapid equilibrium between N′ and C | $\frac{dN'C'}{dt} = b[HRN]$ | Non-first-order, similar to BII |
| C. | $HR + N \underset{K_{RN}}{\rightleftharpoons} HRN + C \xrightarrow[a]{} (HRNC \rightleftharpoons HRN'C') \xrightarrow[b]{} HR + N'C'$ | $\frac{dN'C'}{dt} = a[HRN][C]$ | Non-first-order unless $N_T \gg K_{RN}$ |

Symbols are: $[R_T]$, total receptor concentration; $[N_T]$, total guanyl nucleotide binding protein concentrations to which p[NH]ppG is bound; $[C_T]$, total catalytic unit concentration; a, b, and c denote apparent "net" rate constants, which represent unidirectional fluxes in the direction of activation; $K_{RN}$, equilibrium dissociation constant between the receptor and the p[NH]ppG bound guanyl nucleotide binding protein, N; $K_{NC}$, equilibrium dissociation constant between the p[NH]ppG bound guanyl nucleotide binding protein N and the catalytic subunit; [N′], the concentration of the activated species of N; [N′C′], the concentration of the activated catalytic subunit. Details in ref. 14.

the rate-limiting step is the R–N interaction and N′ and C are in rapid equilibrium, predicts that at C concentrations below $K_{NC}$, deviations from first-order kinetics will be observed. The deviation from first order increases as the reaction progresses. In the turkey erythrocyte system, such deviations have not been observed. Therefore, in order to conform to this model, one must assume that at all C concentrations examined, $[C] \gg K_{NC}$, thus reducing model BIV to model A. (*iii*) Another possibility (model C) is that N is preferably coupled in its inactive form to R and in its active form N′ to C, and the rate limiting step is the interaction between the complex H·R·N and C. In the general case, such a model predicts a kinetic pattern which deviates from first order, in which the rate of activation depends on N. In a special case, where $[N_T] \gg [R_T] + [C_T]$ and where $[N_T] \gg K_{RN}$, a first-order kinetic pattern is observed. However, this latter possibility can be rejected because limiting N reduces the extent of activation (Figs. 1 and 6), namely, $[N_T]$ is not in excess of $[C_T]$. In conclusion, all of our data support model A, in which N and C are tightly associated during the entire course of activation, as the model of choice.

**The Nature of the Rate-Limiting Step.** The rate-limiting step is not GDP dissociation (Fig. 5) from the inactive unit N. It also does not involve the interaction of N′ with C or the transition from N to N′. Our results strongly suggest that the rate limiting step involves the interaction between HR and the nucleotide binding protein N, in which the intermediate does not accumulate:



$$HR + N{\cdot}C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H{\cdot}R{\cdot}N{\cdot}C \xrightarrow{k_3} H{\cdot}R{\cdot}N'C' \xrightarrow{k_4} HR + N'{\cdot}C', \quad [2]$$

where $(k_2 + k_3) \gg k_1$. The bimolecular rate constant $k$ (Eq. 1) is given by:

$$k = \frac{k_1 k_3}{k_2 + k_3}, \quad [3]$$

and the pseudo-first-order rate constant of activation is:

$$k_{on} = \frac{k_1 k_3}{k_2 + k_3}[R_T]\frac{[H]}{K_H + [H]} \quad [4]$$

Therefore, we have revealed the nature of $k$ in Eq. 1. Examination of model BII (Table 1) reveals that if $[N_T]$ were much larger than $[R_T]$, the N-to-N′ conversion would become rate limiting. Such conditions have apparently been achieved in reconstitution experiments (6). At high N-to-R ratios, the amount of N′ produced is independent of $[N_T]$, and even 5 min of incubation yield initial rates (6). By contrast, in the native turkey membrane, the *entire* activation process is complete within 3 min of incubation, corresponding to the low ratios of N to R used in the experiments performed on reconstituted systems.

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1. Schramm, M. & Rodbell, M. (1975) *J. Biol. Chem.* **250,** 2232–2237.
2. Sevilla, N., Steer, M. L. & Levitzki, A. (1976) *Biochemistry* **15,** 2493–3499.
3. Cassel, D., Lefkovitz, H. & Selinger, Z. (1977) *J. Cyclic Nucleotide Res.* **3,** 393–406.
4. Cassel, D. & Selinger, Z. (1977) *Biochem. Biophys. Res. Commun.* **77,** 868–874.
5. Tolkovsky, A. M. & Levitzki, A. (1978) *Biochemistry* **17,** 3795–3810.
6. Citri, Y. & Schramm, M. (1980) *Nature (London)* **287,** 297–300.
7. De Lean, A., Stadel, J. M. & Lefkowitz, R. J. (1980) *J. Biol. Chem.* **255,** 7108–7117 (and refs. therein).
8. Steer, M. L. & Levitzki, A. (1975) *J. Biol. Chem.* **250,** 2080–2084.
9. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.* **58,** 541–548.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
11. Pfeuffer, T. & Helmreich, J. M. (1975) *J. Biol. Chem.* **250,** 867–876.
12. Blume, A. J. & Foster, C. J. (1976) *J. Biol. Chem.* **251,** 3399–3404.
13. Cassel, D. & Selinger, Z. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4155–4159.
14. Tolkovsky, A. M. & Levitzki, A. (1981) *J. Cyclic Nucleotide Res.* **7,** in press.
15. Ross, E. M. (1981) *J. Biol. Chem.* **256,** 1949–1953.
16. Neer, E. J., Echeverria, D. & Knox, S. (1980) *J. Biol. Chem.* **255,** 9782–9789.