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PREFERENTIAL UNCOUPLING BY MANGANESE OF ALPHA ADRENERGIC RECEPTOR MEDIATED INHIBITION OF ADENYLATE CYCLASE IN HUMAN PLATELETS

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SUMMARY

Mn^{++} was found to preferentially uncouple the alpha receptor mediated inhibition of adenylate cyclase in human platelets under conditions where PGE_1 stimulation of the enzyme was preserved. Since epinephrine competition curves with the antagonist radioligand [^{3}H]yohimbine were unchanged by Mn^{++}, it is likely that the Mn^{++} perturbs the interaction of an inhibitory regulatory component with adenylate cyclase rather than with the alpha receptor. The ability of low concentrations of Mn^{++} to selectively uncouple a functionally distinct pathway which mediates inhibition of adenylate cyclase by alpha-adrenergic agonists and guanine nucleotides should provide a useful tool for probing the mechanisms of hormonal regulation of the enzyme's activity.

INTRODUCTION

The interaction of a number of hormones with cell surface receptors leads to activation of adenylate cyclase through the mediation of well-characterized guanine nucleotide regulatory proteins (1). Other hormone and drug receptors, such as the $alpha_2$-adrenergic receptors of human platelets, are involved in the inhibition of adenylate cyclase activity through mechanisms that are less well understood. Guanosine triphosphate (GTP) modulates both the inhibition of adenylate cyclase by epinephrine and the affinity of this agonist for the $alpha_2$ receptors in human platelets (2-5). Therefore, it is likely that a guanine nucleotide regulatory protein is involved in mediating

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epinephrine-induced inhibition of adenylate cyclase in the platelet. A major question has been whether there are distinct nucleotide regulatory components for stimulatory and inhibitory receptors respectively (6).

In an effort to delineate the inhibitory regulatory component we have made use of manganese (Mn^{++}) which is known to impair both isoproterenol and prostaglandin E_1 (PGE_1) stimulated adenylate cyclase (7). For these stimulatory receptors, high concentrations of Mn^{++} interfere with functionally important interactions between guanine nucleotide regulatory proteins and adenylate cyclase without modifying high affinity agonist binding (7). In the present studies, human platelet lysates were used as a model system for an alpha receptor which inhibits adenylate cyclase activity. We have found that this inhibitory response is preferentially sensitive to perturbation by low concentrations of Mn^{++}. It was thereby possible to successfully distinguish functionally separable pathways of the system that are involved respectively in the activation and inhibition of adenylate cyclase.

METHODS

Platelet lysates were prepared as previously described (8) and resuspended in a buffer containing 50 mM Tris HCl and 1 mM EDTA (pH 7.5). Adenylate cyclase assays were done as previously described except the final incubations contained 2 mM dithiothreitol, 0.4 mM EDTA, 0.12 mM ATP and indicated ions in excess of the EDTA (9). Radioligand binding assays using [^{3}H]yohimbine (81 Ci/mmol, New England Nuclear Corp.) were performed in a total volume of 1.0 ml containing 0.1 ml of platelet lysates, 1 mM dithiothreitol, [^{3}H]yohimbine (1.5-2 nM), 50 mM Tris HCl (pH 7.5) and ions as indicated. [^{3}H]Yohimbine labels $alpha_2$ receptors in human platelets (10).

RESULTS

The effects of Mn^{++} on the stimulation and inhibition of adenylate cyclase activity in human platelet lysates are shown in Figure 1. In the absence of added divalent cations there was no adenylate cyclase activity. Increasing concentrations of Mn^{++} in the form of $MnCl_2$ caused progressive increases in PGE_1-stimulated adenylate cyclase activity over the range of 0.05 mM to 1.6 mM. Indeed, appropriately chosen Mn^{++} concentrations could support PGE_1-stimulated activity essentially equal to the maximal activity observed in

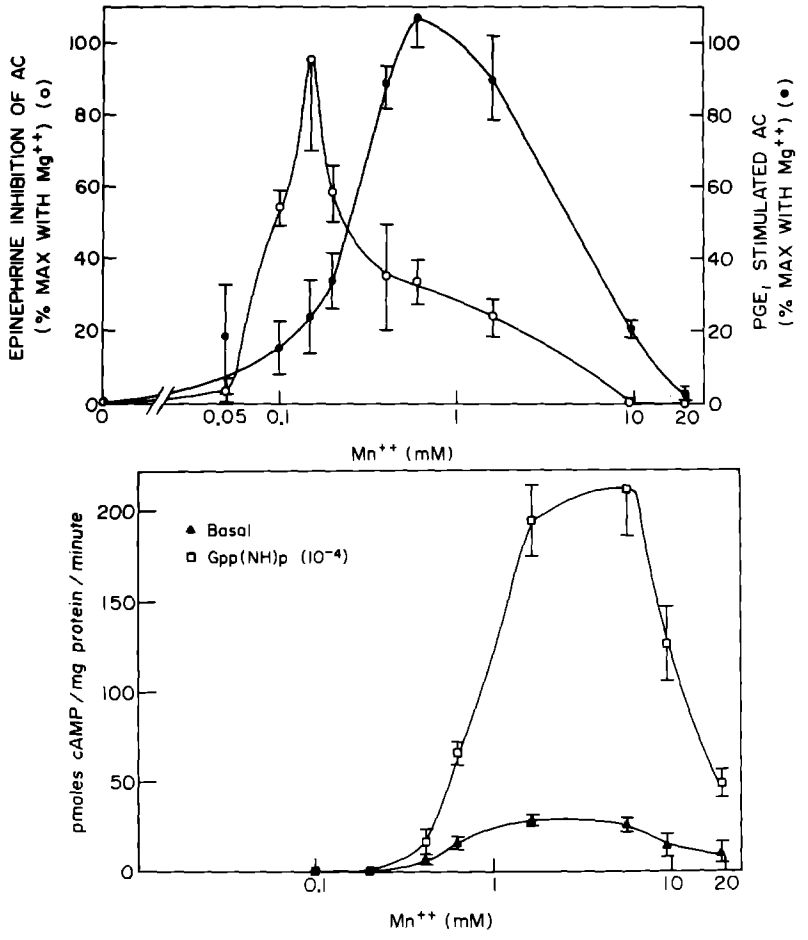

Figure 1. Adenylate cyclase activity in human platelet lysates as a function of $[Mn^{++}]$.
Upper panel. The data for PGE_1 (10 μM) stimulated activity were normalized to the amount of PGE_1 stimulated activity found in the presence of 3.6 mM $MgCl_2$. The epinephrine induced inhibition of adenylate cyclase refers to the inhibition of PGE_1 stimulated activity by 0.1 mM epinephrine. The inhibition in the presence of 3.6 mM $MgCl_2$ (generally 40-50% of activity in the presence of PGE_1 alone) was taken to represent "100% inhibition" to which the values (%) in the presence of Mn^{++} were normalized. The data points are the means ± S.E.M.of 3-6 experiments, performed in triplicate: In the presence of $MgCl_2$, activities were: basal, 15 ± 1; and PGE_1 stimulated, 287 ± 20 pmoles cAMP/mg protein/minute.
Lower panel. The activation of adenylate cyclase by Gpp(NH)p (0.1 mM) is indicated (mean of three experiments). Gpp(NH)p does not appreciably activate adenylate cyclase until $[Mn^{++}] \geq 600$ μM. In these experiments, in the presence of 3.6 mM $MgCl_2$ Gpp(NH)p stimulated activity was 23 ± 2 pmoles cAMP/mg protein/minute.

the presence of optimal $MgCl_2$. However, the PGE_1-stimulated adenylate cyclase activity was impaired at $[Mn^{++}] > 1.6$ mM.

The concentration-dependent effects of Mn^{++} on alpha-adrenergic receptor induced inhibition of PGE_1-stimulated adenylate cyclase are quite different. Much lower concentrations of Mn^{++} (in the range of 0.15 mM) supported maximal epinephrine mediated inhibition of PGE_1-stimulated adenylate cyclase. However, in the range of $[Mn^{++}]$ of 0.4 to 1.6 mM the ability of epinephrine to inhibit adenylate cyclase was markedly impaired, even though PGE_1 stimulation remained near maximal (Figure 1,upper panel). Thus lower concentrations of Mn^{++} preferentially "uncouple" the inhibitory alpha-adrenergic receptor mediated response compared with the stimulation of adenylate cyclase by PGE_1.

The possibility of functionally distinct regulatory pathways for inhibition and stimulation of adenylate cyclase was further examined with the non-hydrolyzable guanine nucleotide analog Gpp(NH)p. In the presence of $MgCl_2$, Gpp(NH)p causes little stimulation of adenylate cyclase (11). An explanation for this observation might be that Gpp(NH)p activates both the inhibitory and stimulatory regulatory components with the balance of effects leading to only a small increase in adenylate cyclase activity. This hypothesis was tested with the use of Mn^{++} (Fig. 1, lower panel). Gpp(NH)p (0.1 mM) did not elicit appreciable adenylate cyclase activation until $[Mn^{++}]$ exceeded 600 μM. Therefore, marked Gpp(NH)p activation of adenylate cyclase requires concentrations of Mn^{++} that severely impair the interaction of the inhibitory regulatory component with adenylate cyclase as judged from the loss of alpha receptor mediated inhibition of the enzyme. At much higher Mn^{++} concentrations, Gpp(NH)p no longer activated adenylate cyclase since the interaction of the stimulatory component with adenylate cyclase is also impaired. This decline in Gpp(NH)p stimulated activity parallels the fall in PGE_1 stimulated activation. In the presence of Gpp(NH)p, PGE_1 caused no further activation of adenylate cyclase (data not shown).

To explore the mechanism responsible for the effect of Mn^{++} on the uncoupling of the alpha receptor mediated inhibition of adenylate cyclase,

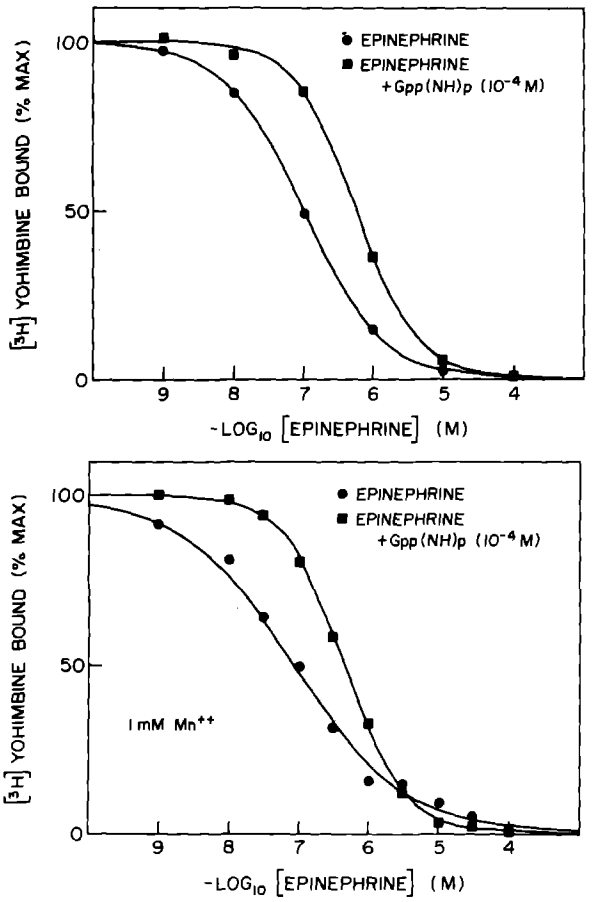

Figure 2 Competition curves of epinephrine and [^{3}H]yohimbine in the presence and absence of Mn^{++} in human platelet lysates. This experiment is representative of two, performed in duplicate. The upper panel represents the binding of epinephrine in the presence of 6 mM $MgCl_2$ whereas in the lower panel the competition curves were done in the presence of 1 mM $MnCl_2$. In both cases, epinephrine affinities were similarly affected by Gpp(NH)p. The curves were computer drawn according to a four paramenter logistic equation as previously described (12).

experiments were performed to directly measure the interaction of epinephrine with the alpha receptor in the platelet lysates. Competition curves of epinephrine with [^{3}H]yohimbine in the presence of either $MgCl_2$ (6 mM) or $MnCl_2$ (1 mM) were constructed (Figure 2). At this concentration of Mn^{++} which severely impairs alpha receptor mediated inhibition of adenylate cyclase, there is no effect on the affinity of the alpha receptor for epinephrine. Also, guanine nucleotides cause a similar reduction in agonist affinity in the

presence or absence of Mn^{++}. These results indicate that the effect of Mn^{++} to uncouple alpha receptor mediated inhibition of the cyclase is not due to a perturbation in agonist-receptor interactions.

DISCUSSION

These results demonstrate that Mn^{++} preferentially uncouples the alpha receptor of human platelets from adenylate cyclase inhibition compared with PGE_1 stimulated activity. At a concentration of Mn^{++} that markedly impairs alpha receptor-mediated inhibition of adenylate cyclase, there is no effect on the alpha receptor's affinity for epinephrine nor its regulation by guanine nucleotides. High affinity, guanine nucleotide sensitive binding of agonists to the alpha receptor of human platelet lysates reflects their mode of action. For example, the extent to which an agonist's overall affinity is reduced by guanine nucleotides is directly related to the agonist's intrinsic activity for inhibition of the enzyme (4). It has been suggested, in analogy with the beta-adrenergic receptor, that this high affinity binding reflects interaction of the receptor with another membrane component which may be a guanine nucleotide regulatory protein (5). Therefore, the absence of an effect of Mn^{++} on epinephrine competition curves suggests that the alpha receptor is still capable of coupling to this component even under conditions where adenylate cyclase inhibition is largely abolished. In frog erythrocytes which contain exclusively stimulatory receptors, high concentrations of Mn^{++} do not impair beta adrenergic receptor-regulatory component interactions under conditions where adenylate cyclase activation is abolished (7).

The present data suggest the model for hormonal regulation of adenylate cyclase shown in Figure 3. Inhibitory (e.g. $alpha_2$) and stimulatory (e.g. PGE_1) receptors interact with and promote the association of distinct nucleotide regulatory components with the catalytic moiety of adenylate cyclase. Gpp(NH)p is capable of activating both pathways and thus under ordinary circumstances its overall effect is the net result of both

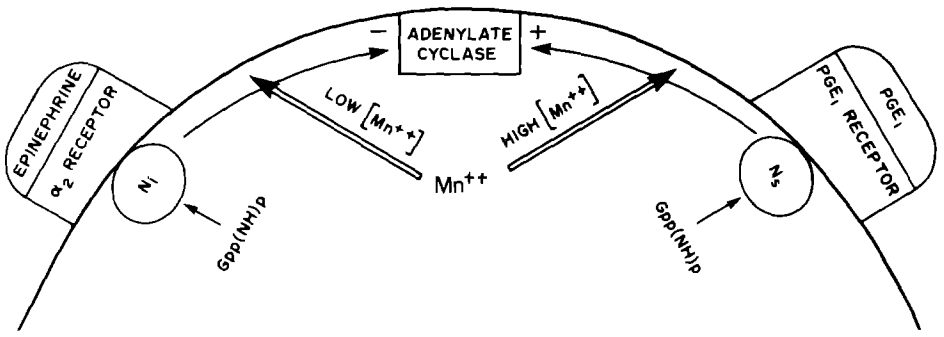

Figure 3 Model of Mn^{++} interactions with guanine nucleotide regulatory components of hormone sensitive adenylate cyclase. Epinephrine binding to the alpha$_2$ receptor activates the inhibitory guanine nucleotide regulatory component (N_i) which inhibits adenylate cyclase activity. PGE_1 binding to the PGE_1 receptor activates the stimulatory guanine nucleotide regulatory component (N_s) which stimulates adenylate cyclase. Both of these components are activated by Gpp(NH)p. Mn^{++} at low concentrations preferentially impairs the inhibitory interactions of N_i with adenylate cyclase.

stimulation and inhibition, which in the case of the platelet is slight stimulation. The interaction of the inhibitory component with adenylate cyclase is preferentially sensitive to low concentrations of Mn^{++}. Higher concentrations of Mn^{++} also uncouple stimulatory receptors. Thus Gpp(NH)p only maximally stimulates the enzyme when the inhibitory pathway is blocked by low $[Mn^{++}]$. Distinct regulatory components in human platelets are also suggested by the observation that higher concentrations of GTP are required to support alpha receptor mediated inhibition of adenylate cyclase than PGE_1 stimulated activity (3).

The utilization of Mn^{++} in the fashion described here should provide a powerful tool for functionally dissecting the stimulatory and inhibitory regulatory components of adenylate cyclase in the host of cells containing both stimulatory and inhibitory receptors.

REFERENCES

1. Ross, E.M. and Gilman, A.G. (1980) Ann. Rev. Biochem. 49,533-564.
2. Jakobs, K.H., Saur, W. and Schultz, G. (1978) FEBS Lett. 85,167-170.
3. Steer, M.L. and Wood, A. (1979) J. Biol. Chem. 254,10791-10797.

4. Tsai, B.S. and Lefkowitz, R.J. (1979) Mol. Pharmacol. 16,61-68.
5. Hoffman, B.B., Mullikin-Kilpatrick, D. and Lefkowitz, R.J. (1980) J. Biol. Chem. 255,4645-4652.
6. Rodbell, M. (1980) Nature. 284,17-22.
7. Limbird, L.E., Hickey, A.R. and Lefkowitz, R.J. (1979) J. Biol. Chem. 254,2677-2683.
8. Hoffman, B.B., Michel, T., Mullikin-Kilpatrick, D., Lefkowitz, R.J., Tolbert, M.E.M., Gilman, H. and Fain, J.N. (1980) Proc. Natl. Acad. Sci. U.S.A. 77,4569-4573.
9. Newman, K.D., Williams, L.T., Bishopric, N.H. and Lefkowitz, R.J. (1978) J. Clin. Invest. 61,395-402.
10. Motulsky, H.J., Shattil, S.J. and Insel, P.A. (1980) Biochem. Biophys. Res. Comm. 97,1562-1570.
11. Tsai, B.S. and Lefkowitz, R.J. (1979) Biochim. Biophys. Acta. 587,28-41.
12. De Lean, A., Munson, P.J. and Rodbard, D. (1978) Am. J. Physiol. 4,E97-E102.