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Magnesium Regulation of the *Beta*-Receptor-Adenylate Cyclase Complex

I. Effects of Manganese on Receptor Binding and Cyclase Activation

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SUMMARY

The ability of manganese ion to interact with specific sites for free Mg^{2+} on the *beta*-adrenergic receptor-adenylate cyclase complex has been investigated in murine S49 lymphoma cell variants possessing specific defects in receptor-cyclase coupling. Free Mg^{2+} activates adenylate cyclase in the S49 *unc* variant in a manner identical with the activation seen in wild-type S49 cells. *Unc* is defective in coupling of *beta*-adrenergic receptors to the adenylate cyclase catalytic complex, and Mg^{2+} cannot alter agonist affinity for the *beta*-receptor. In contrast, Mg^{2+} is able to alter agonist affinity for *beta*-receptors of the S49 variant H21a and of the HC-1 hepatoma line. These cell lines have functional receptor and coupling protein, but the cyclase catalytic unit is either uncoupled or inactive, respectively. In wild-type S49 cell membranes, free Mn^{2+} increases the V_{max} of the fluoride-stimulated adenylate cyclase activity but decreases the V_{max} of hormone/GTP-stimulated activity. Free Mn^{2+} also increases the V_{max} in the *cyc*$^-$ S49 variant which lacks functional coupling protein but has a functional cyclase catalytic unit. Free Mn^{2+} has no effect on the K_m for the substrate $MnATP^{2-}$ in either wild-type or *cyc*$^-$ S49 cell membranes. Furthermore, free Mn^{2+} blocks the effect of Mg^{2+} to increase agonist affinity for *beta*-adrenergic receptors but has no effect by itself. These data are most easily interpreted as indicating the presence of two independent metal-binding sites on the receptor-cyclase complex, one site on the coupling protein(s) mediating metal ion effects on agonist affinity for the receptor and a second site on the cyclase catalytic subunit mediating metal ion activation of cyclase activity.

INTRODUCTION

Free Mg^{2+} specifically modulates adenylate cyclase activity from numerous cells and species regardless of the hormonal agent responsible for activating or inhibiting the enzyme. This evidence has recently been reviewed (1). The action of the hormone is generally to decrease the K_a for free Mg^{2+} activation from about 1–3 mM to 0.1 mM (1–5). This "basal" K_a for free Mg^{2+} of 1–3 mM is substantially higher than the intracellular concentration of free Mg^{2+}, which is less than 0.3 mM in S49 cells[3] and generally about 0.3–0.5 mM in other cell types (6–8). Thus, the effect of hormone may be to alter the activation characteristics of adenylate cyclase to permit Mg^{2+} activation to occur. In contrast, although GTP is undeniably an absolutely required cofactor for receptor activation of adenylate cyclase, the intracellular concentration of GTP (>100 μM) is far in excess of the amount required for maximal cyclase activation. Moreover, depletion of intracellular GTP has only minimal effects on hormonal responsiveness of the receptor-cyclase complex (9–11). Thus, the properties of Mg^{2+} activation of the receptor-cyclase complex and the physiological data on cellular Mg^{2+} suggest that free Mg^{2+} is a potential regulatory agent of the receptor-cyclase complex.

We have initiated a series of investigations (5, 12–15) to determine the location and properties of the Mg^{2+} site(s) active on the receptor-cyclase complex. The data presented in this and the following paper (17) suggest that two distinct Mg^{2+} sites exist on the receptor-cyclase complex of the S49 cell and that Mn^{2+} and Sc^{3+} each interact with these two sites differently.

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METHODS

Cell culture. S49 lymphoma cells were grown in suspension culture and harvested, and membrane fractions were prepared as previously described (5, 18). HC-1 cells were grown in Dulbecco's modified essential medium with 10% heat-inactivated horse serum in plastic tissue culture dishes under conditions identical with those for S49 cells. A membrane fraction from HC-1 cells was obtained as for S49 cells, except for preparation of the initial homogenate. Dishes containing HC-1 cells were rinsed twice with phosphate-buffered isotonic saline [10 mM KPO_4 (pH 7.4) and 150 mM NaCl] and then allowed to swell for approximately 1 min in homogenization buffer [20 mM Na-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 8.0 at 4°), 2 mM $MgSO_4$, and 1 mM EDTA]. The cells were then scraped from the dishes with a Teflon-coated spatula and homogenized with 10 strokes in a tight-fitting Dounce homogenizer. From this point, preparation of S49 and HC-1 membranes was identical (18).

Assays. Adenylate cyclase was measured using [α-^{32}P] ATP (Amersham) as previously described (5), with isolation of ^{32}P-labeled cyclic AMP by the method of Salomon *et al.* (19). Cyclase assays were generally performed for 20–30 min. However, when Mn^{2+} was present, assay times were 10 min to minimize possible Mn^{2+}-catalyzed oxidation. Adrenergic receptors were measured as previously described (5, 7), using [^{125}I]IHYP.[4] $MnCl_2$ stock solutions were made 1 mM in HCl, aliquoted into test tubes, and stored frozen. This prevented oxidation of Mn^{2+} to higher valence states, which was evidenced by a brown precipitate within 1 week if stock solutions were kept on the bench or in the refrigerator. All assays contained 1 mM ascorbate to help slow Mn^{2+}-catalyzed oxidation. Because of the longer incubation times required in the binding assays, agonist competition for *beta*-adrenergic receptors was usually performed by using (±)-metaproterenol rather than (−)-isoproterenol. Both compounds are full agonists in S49 cells, but the former is a resorcinol rather than a catechol amine derivative and thus not subject to oxidation. Specific binding was defined with the use of 1 μM (−)-propranolol and was 85% of total binding in S49 membrane preparations. Specific binding in HC-1 varied slightly between preparations, but was generally 50–60% of total binding. In the specific experiments shown, specific binding was about 70% of the total. However, similar results were also obtained with membrane preparations showing lower specific binding.

In all assays shown, "free" Mg^{2+} is a relative calculation and is defined as the difference between total [Mg^{2+}] and the sum of [ATP] and [EDTA]. Any K values for the various cation effects are therefore relative rather than absolute numbers. Since we and others have previously shown that unchelated ATP is not an inhibitor, this definition of "free" Mg^{2+} introduces no significant error in the range of Mg^{2+} and ATP concentrations used (see further discussion of this point in refs. 1, 2, and 5).

[4] The abbreviations used are: IHYP, iodohydroxybenzylpindolol; R, receptor; G/F and C, the regulatory and catalytic components of adenylate cyclase, respectively.

RESULTS

Because cyclic AMP is cytocidal in S49 lymphoma cells, a number of variant clones have been isolated that possess specific defects in proteins of the receptor-cyclase complex (for review, see ref. 20). In the *unc* variant, the metal/guanine nucleotide-coupling proteins (G/F) are partially defective and can interact only with the catalytic subunit (C) and not the *beta*-receptor (R). We have previously shown that Mg^{2+} is unable to alter agonist affinity for *beta*-receptors in this variant (5), which suggests that the Mg^{2+} site responsible for this effect is not resident on R. Mg^{2+} specifically increases the V_{max} for both basal and fluoride-stimulated activities in the *unc* variant (Fig. 1B) in a manner qualitatively identical with that observed in wild-type cells (Fig. 1A). In neither case is the K_m for $MgATP^{2-}$ affected by Mg^{2+} concentration. This suggests that a Mg^{2+} site resident on either G/F or C is responsible for activation of adenylate cyclase and that this site is still expressed in the *unc* variant.

The Mg^{2+} site responsible for modulation of receptor affinity (5, 21) can be investigated by use of the HC-1 hepatoma cell line and the S49 variant line H21a. HC-1 has functional *beta*-adrenergic receptors and G/F proteins but does not have a functional C (22). The H21a line has defective G/F proteins which associate with the *beta*-receptor appropriately but cannot associate with the catalytic subunit (23). Figure 2 indicates that Mg^{2+} ion can increase *beta*-receptor affinity for agonist in membranes from both of these lines. The magnitude of the Mg^{2+} effect in HC-1 membranes appears slightly smaller than that seen in wild-type S49 cells but is equal to or larger than that seen with Mg^{2+} in turkey erythrocyte membrane preparations (15). The shallow slope of the agonist inhibition curve in HC-1 (Hill coefficient of about 0.4) is probably the result of a very high proportion of the low-affinity form of the *beta*-receptor as described by Kent and colleagues (24). This might be due to some alteration in the equilibrium between R and G/F because of the absence of C. However, Ross and colleagues (22) have shown that both R and G/F in HC-1 cells are fully functional. The results with the H21a variant are quite similar to those seen in wild-type S49 cells. Thus the data indicate that a complex of R plus G/F in the absence of functional C contains a Mg^{2+} site capable of regulating receptor affinity for agonist.

These two functions of Mg^{2+}, modulation of receptor affinity for agonist and activation of adenylate cyclase, were further investigated by studying the effects of potential Mg^{2+} analogues or antagonists. This paper presents data obtained with the use of Mn^{2+}, whereas the following paper (17) discusses the differential effects of Sc^{3+} versus La^{3+}. Figure 3 shows Mn^{2+} activation dose-response curves for fluoride- and (−)-isoproterenol/GTP-activated adenylate cyclase in wild-type S49 cells. Mn^{2+}, like Mg^{2+}, activates fluoride-stimulated cyclase activity by increasing the V_{max}. The K_m for $MnATP^{2-}$ is not altered by increasing free Mn^{2+} concentration. A similar effect of Mn^{2+} on the V_{max} but not the K_m is seen when basal activity is measured (data not shown). In sharp contrast to the effect of Mn^{2+} on fluoride-stimulated activity, free Mn^{2+} inhibits (−)-isoproterenol/GTP-stimulated cyclase activity. This inhibition is again an effect

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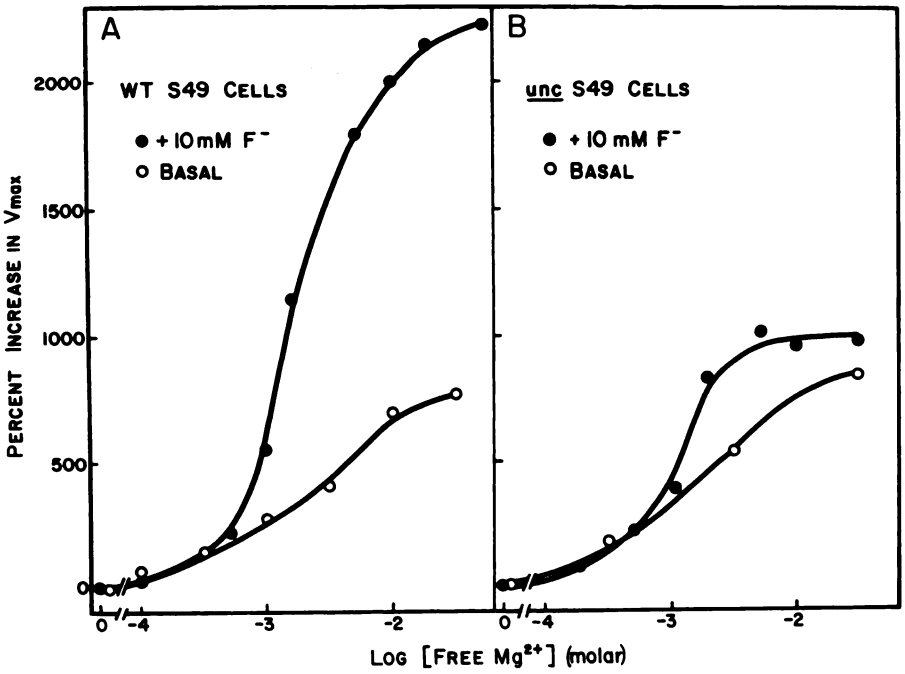

FIG. 1. *Mg^{2+} activation of adenylate cyclase in membranes from wild-type and unc S49 cells*

The data are presented as the percentage increase in the V_{max} in membrane preparations from wild-type (A) and *unc* (B) S49 cells. They are plotted as a function of the V_{max} at various concentrations of free Mg^{2+} as compared with the V_{max} at "zero" free Mg^{2+} for both basal (○) and NaF-stimulated (●, 10 mM) activity. The V_{max} was derived from double-reciprocal plots of activity at several concentrations of free Mg^{2+}. The K_m did not change at any concentration of Mg^{2+} and was 50 ± 20 μM in both wild-type and *unc* membranes in the experiments shown. This slight variation in K_m showed no dependence on $[Mg^{2+}]$. V_{max} and K_m values were determined by least-squares analysis; in the experiments shown, the lines had an average correlation coefficient as follows: A, Basal (○) = 0.996 ± 0.002 SD (n = 7); NaF (●) = 0.991 ± 0.005 SD (n = 9). B, Basal (○) = 0.993 ± 0.006 SD (n = 4); NaF (●) = 0.988 ± 0.007 SD (n = 8). The maximal values of V_{max} were 12, 740, 17, and 235 pmoles/min/mg of protein for basal and NaF-stimulated activities in A and B, respectively.

on the V_{max} rather than the K_m. The K_a for Mn^{2+} activation of fluoride-stimulated cyclase (1 mM) is essentially the same as its K_i for inhibition of (−)-isoproterenol/GTP-stimulated cyclase (2 mM). Since both inhibition and activation affect the V_{max} and have similar dose dependency, Mn^{2+} is presumably acting at the same site

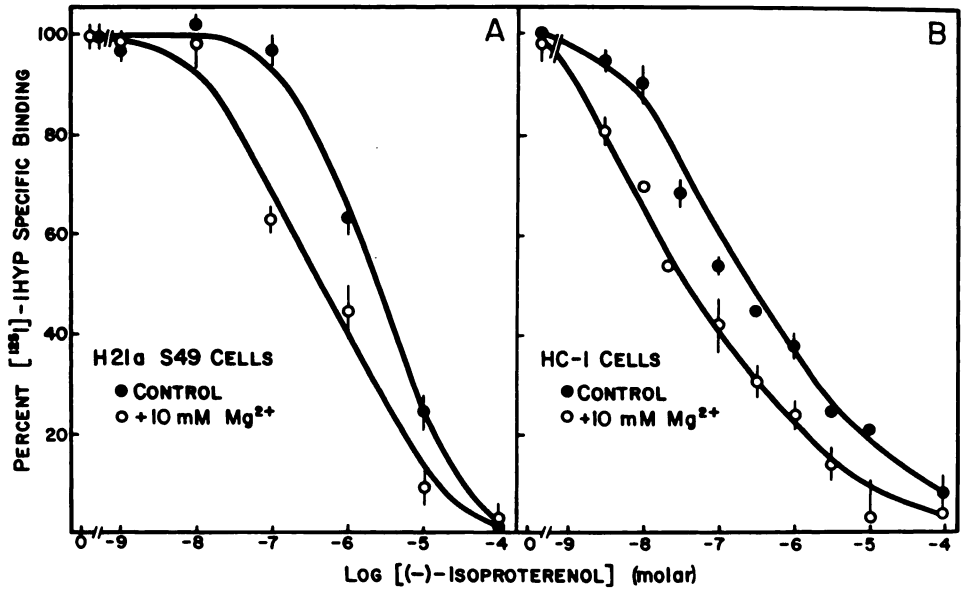

FIG. 2. *Effect of Mg^{2+} on receptor affinity for agonist in H21a S49 cells and HC-1 cells*

Specific binding of [^{125}I]IHYP was measured in the absence (●) and the presence (○) of 10 mM free Mg^{2+} in membranes from the S49 variant H21a cell line (A) or from HC-1 cells (B) as described (5). Maximal specific binding in H21a membranes was 1050 ± 50 cpm at a protein concentration of 25 μg/ml and was 75% of total binding in the experiment shown. In HC-1 membranes, the corresponding data were 1120 ± 60 cpm, 35 μg/ml, and 70%.

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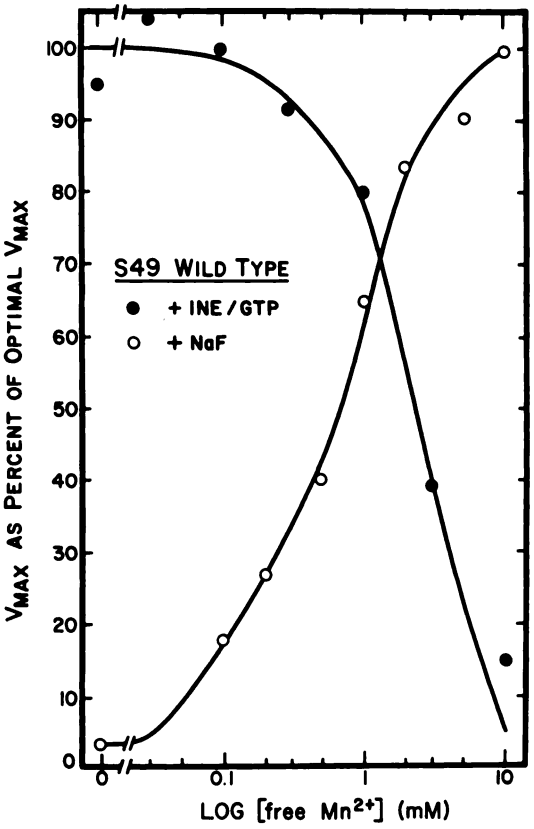

FIG. 3. *Effect of free Mn^{2+} on V_{max} of NaF- and (−)-isoproterenol/GTP-stimulated adenylate cyclase in wild-type S49 cells*

Adenylate cyclase was measured as described under Methods. The data are plotted as a function of the maximal V_{max} for each condition of stimulation, which were 980 (at 10 mM free Mn^{2+}) and 825 (at 0.1 mM free Mn^{2+}) pmoles/min/mg of protein for NaF- (○) and (−)-isoproterenol/GTP-stimulated (●) activities, respectively. V_{max} and K_m values were derived from double-reciprocal plots of activity at various concentrations of free Mn^{2+} as described in legend to Fig. 1. The K_m values in these experiments were 62 ± 20 μM and 96 ± 20 μM for the two stimulatory conditions. This variation in K_m did not show any dependence on the concentration of free Mn^{2+}. Correlation coefficients of the lines used to derive V_{max} were 0.998 ± 0.005 SD (n = 8) and 0.999 ± 0.003 SD (n = 7) for NaF- and (−)-isoproterenol/GTP-stimulated cyclase activities, respectively. Concentrations of stimulatory agents were 10 mM NaF, 10 μM (−)-isoproterenol, and 10 μM GTP.

in both cases. Alternatively, this inhibition of (−)-isoproterenol/GTP-stimulated activity by Mn^{2+} could be due to oxidation of the catecholamine or to a shift in the dose-response curve for agonist. That this is not the case is shown in Fig. 4 for (−)-isoproterenol in the presence of 1 mM ascorbate. Similar data were obtained using the non-catechol *beta*-agonist (±)-metaproterenol in the absence of ascorbate (data not shown).

The ability of Mn^{2+} to alter receptor affinity for agonist in S49 cells was also determined. Figure 5 shows that Mn^{2+} alone does not alter the agonist competition curve of metaproterenol for [^{125}I]IHYP binding. Furthermore, Mn^{2+} actually inhibits Mg^{2+} since it is able to block the increase in receptor affinity for agonist induced by Mg^{2+} (Fig. 5).

The S49 cell *cyc*$^-$ variant lacks functional G/F and does not exhibit significant $MgATP^{2-}$-dependent adenylate cyclase activity. When $MnATP^{2-}$ is used, adenylate cyclase activity is apparent but cannot be stimulated by (−)-isoproterenol/GTP, 5′-guanylyl imidodiphosphate, fluoride, or cholera toxin (20, 25). $MgATP^{2-}$-dependent cyclase activity can be reconstituted by addition of purified G/F. The reconstituted activity exhibits all properties of wild-type S49 adenylate cyclase activity, including hormonal stimulation (26). Figure 6 shows that the $MnATP^{2-}$-dependent activity in *cyc*$^-$ is activated by free Mn^{2+}. Mn^{2+} does so by specifically increasing the V_{max}; the K_m for $MnATP^{2-}$ is unaltered. Adequate kinetic data from *cyc*$^-$ cells has proved extremely difficult to obtain. For example, a decrease in the V_{max} at higher Mn^{2+} concentrations is evident in the experiment shown. This decrease is variable and not always seen. However, although the experiment shown cannot be said to be representative in a quantitative sense, qualitatively free Mn^{2+} consistently activates adenylate cyclase activity in *cyc*$^-$ preparations. The data suggest that the ability of Mn^{2+} to activate cyclase in *cyc*$^-$ cells is due to a site for free divalent cation on C, the catalytic subunit.

DISCUSSION

The data presented above confirm our previous results (5) and those of Iyengar and Birnbaumer (27, 28) demonstrating the existence of a divalent cation site(s) on the *beta*-receptor-adenylate cyclase complex of the S49 cell.

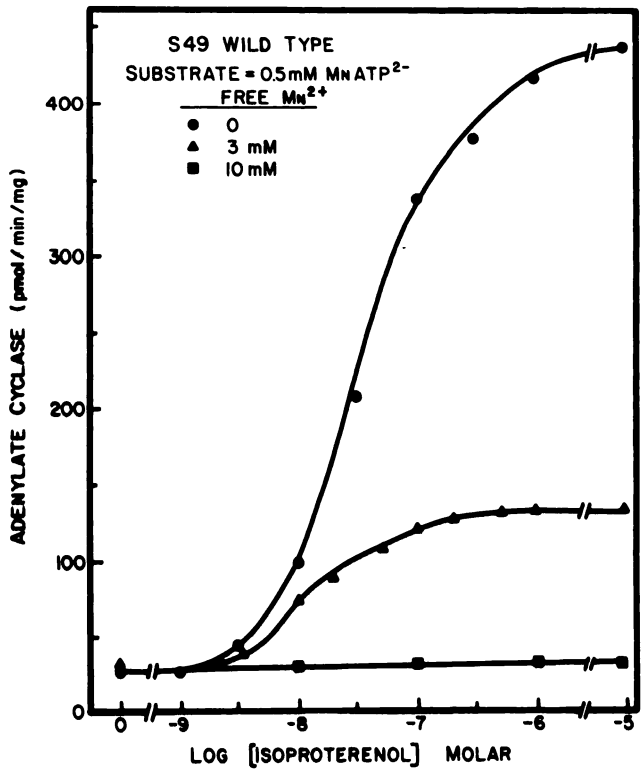

FIG. 4. *Effect of free [Mn^{2+}] on the dose-response curve for (−)-isoproterenol stimulation of adenylate cyclase in wild-type S49 cells*

Adenylate cyclase activity was measured in the presence of 0.5 mM $MnATP^{2-}$, 0.1 mM $MnGTP^{2-}$, and the agonist concentrations shown in the absence (●) and in the presence of 3 mM (▲) or 10 mM (■) free Mn^{2+}. Ascorbate was present at 1 mM. Essentially similar results are seen in the absence of ascorbate with replacement of (−)-isoproterenol by the non-catechol *beta*-agonist, (±)-metaproterenol (data not shown).

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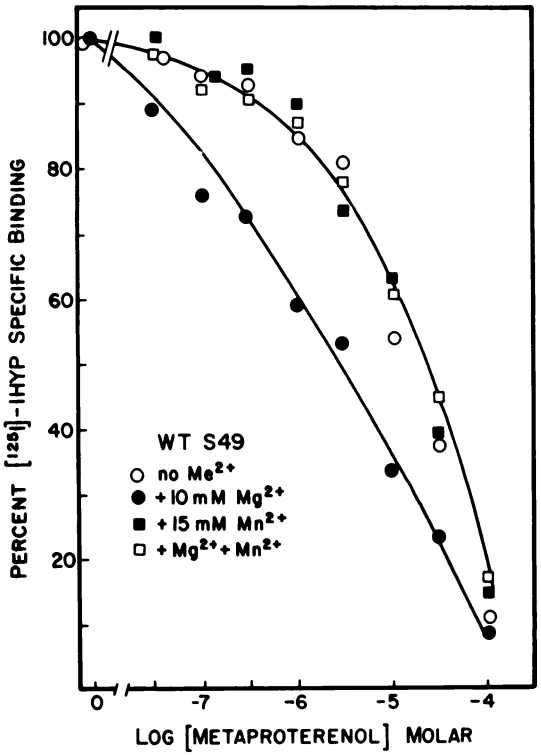

FIG. 5. *Mn^{2+} inhibition of Mg^{2+} modulation of beta-adrenergic receptor affinity for agonist*

Specific binding of [^{125}I]IHYP was measured at various concentrations of the *beta*-agonist (±)-metaproterenol as described (5). Binding was determined in the presence of 0.1 mM EDTA and the indicated divalent cation: no divalent cation (○), 10 mM Mg^{2+} (●), 15 mM Mn^{2+} (■), and both Mg^{2+} and Mn^{2+} (□). Maximal specific binding was 3470 ± 230 cpm at 20 μg of protein per milliliter and was 88% of total binding in this experiment. Binding at each (±)-metaproterenol concentration was determined in triplicate with an average variation of less than 3%. The presence of Mg^{2+} (5) and/or Mn^{2+} at the concentrations shown had no effect on total or nonspecific binding of (±)-metaproterenol. However, concentrations of Mn^{2+} higher than about 20 mM increase both total and nonspecific binding.

Furthermore, they show that Mn^{2+} ion has effects different from those of Mg^{2+} on both receptor binding and cyclase activation and in some circumstances appears to be a Mg^{2+} antagonist. These data suggest that two distinct binding sites exist for free Mg^{2+}, one on C and one on G/F.

The existence of one (or more) metal sites is shown by the ability of both Mg^{2+} (ref. 5; Fig. 1) and Mn^{2+} (Figs. 3 and 6) to increase the V_{max} of adenylate cyclase without alteration of K_m. These experiments were designed to distinguish metal ion activation from inhibition by free ATP. This point has been discussed by several authors previously (2, 3) as well as ourselves (1, 5), and there seems little question at this point that kinetic mechanisms such as shown in this and previous papers are the result of enzyme activation by free divalent cation.

The metal site responsible for modulation of receptor affinity for agonist is apparently not on the receptor itself, but on the coupling protein(s), G/F. First, the absence of any metal effect on receptor binding in the *cyc*$^-$ variant of S49 cells implies that R alone is insufficient (1, 5). Second, we have previously noted (1) that a guanine nucleotide effect on agonist affinity requires a previous Mg^{2+} effect on agonist affinity. Since Sternweis *et al.* (29) have shown that reconstitution of *cyc*$^-$ with purified G/F from rabbit liver restores the ability of guanine nucleotide to alter receptor affinity for agonist, this also implies reconstitution of a metal effect, carried by G/F. Finally, both HC-1 hepatoma cells and the H21a variant of S49 cells show such a metal effect, but either do not have a functional C or C is uncoupled from G/F. These data together suggest that the metal site is on G/F.

Given this conclusion, the data of Fig. 6 showing that free Mn^{2+} activates cyclase activity in *cyc*$^-$ membranes indicate that a second, independent, metal site exists. This metal site is presumably on C itself, since functional G/F is not present in this variant. However, the G/F moiety is known to consist of at least two polypeptides. The caveat in this interpretation is the possibility that in *cyc*$^-$ (or for that matter in any of the other variants discussed herein), one of the G/F polypeptides is nonfunctional per se whereas the other polypeptide component is present in a competent form but exhibits no apparent activity because of the defect in the other component. In this case, metal ion regulation, rather than distinguishing two independent metal ion sites, is

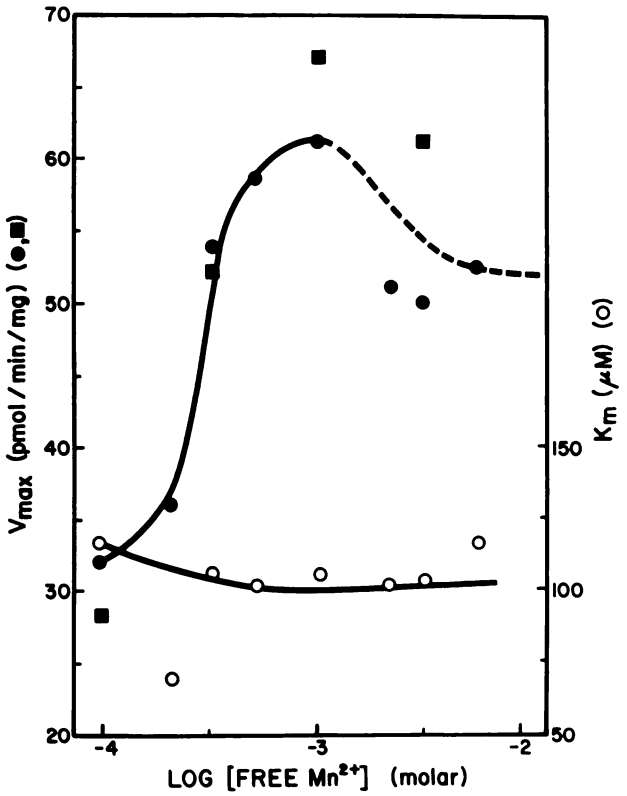

FIG. 6. *Mn^{2+} activation of adenylate cyclase in membranes from cyc$^-$ S49 cells*

Adenylate cyclase activity as a function of free Mn^{2+} was determined as described under Methods. The V_{max} (●) and K_m (○) at each concentration of Mn^{2+} were determined from least-squares analysis of double-reciprocal plots. These plots had an average correlation coefficient of 0.997 ± 0.003 SD (n = 8) in the experiment shown. The V_{max} from an experiment using a different *cyc*$^-$ membrane preparation is also shown for comparison (■); the K_m also did not change in this experiment.

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distinguishing between functions of the peptide components of G/F. However, given the data presented above and the data with Sc^{3+} and La^{3+} in the following paper (17), the most likely interpretation is that there exist two independent metal ion sites.

The putative existence of two distinct Mg^{2+} sites is consistent with the dual effect of Mn^{2+} on adenylate cyclase activity in wild-type S49 membranes. Free Mn^{2+} activates fluoride-stimulated cyclase but inhibits (−)-isoproterenol/GTP-stimulated cyclase. The explanation for this behavior lies ultimately in the fact that GTP alone is a rather poor stimulant of adenylate cyclase in S49 cells. First, Mn^{2+} apparently prevents functional R-G/F interaction, since Mn^{2+} prevents the effect of Mg^{2+} on receptor affinity (Fig. 5), an effect that requires R-G/F interaction (1, 5). Thus, in the presence of Mn^{2+}, (−)-isoproterenol would be unable to promote functional R-G/F interaction and thus stimulate activity. Since the remaining GTP is a weak stimulant in S49 cells, total activity declines to near-basal levels. This decreased level is still activated relative to basal activity at low free Mn^{2+}, but the decline looks similar to a total inhibition of activity since both basal and GTP-stimulated activity are quite low in S49 cells as compared with hormone-stimulated activity. Conversely, since R is not involved in fluoride-stimulated cyclase activity, Mn^{2+} is able to stimulate activity in the presence of fluoride by acting at the site resident on C. Indeed, it is probable that fluoride activation per se requires free metal ion (1). These differential Mn^{2+} effects on various forms of stimulation are compatible with two Mn^{2+} sites as just explained; however, a single Mn^{2+} site on G/F is sufficient to explain the data in Fig. 3 simply by assuming that occupation of the metal site by Mn^{2+} not only prevents functional R-G/F interaction but also activates C via G/F activation. Although this is possible, it seems more reasonable to utilize the strong indication of two distinct sites derived from the data with *cyc*$^-$ cells.

Limbird and colleagues (30) have also used Mn^{2+} to uncouple functionally the hormone-stimulated adenylate cyclase of frog erythrocyte membranes. In the presence of Mn^{2+}, agonist promotes an apparent G/F-dependent increase in the size of R on gel filtration columns. Second, guanyl nucleotide substantially decreases agonist affinity in the presence of Mn^{2+}. This latter result indicates that Mn^{2+} can cause formation of the high-affinity agonist form of the *beta*-receptor in frog erythrocyte membranes. The simplest interpretation of the difference in the results of Limbird *et al.* (30) and ours is that Mn^{2+} is able to act as an antagonist at the G/F site in S49 cell membranes but is an agonist in frog erythrocyte membranes, at least with regard to R-G/F interactions. In either system, however, Mn^{2+} appears to uncouple R-G/F from C. The ability of Mn^{2+} to uncouple hormone stimulation but not metal ion modulation of agonist binding in frog erythrocyte membranes bears similarity to the mutation in the H21a variant of S49 cells in which G/F is functionally coupled to R but not C (21, 23, 30). Thus, although divalent cation modulation of the receptor-cyclase complex is ubiquitous, the precise action of divalent cation may differ somewhat from system to system and therefore must be individually investigated in each model system.

The actual number of sites for free metal and their specific roles can be determined only by extensive purification of the individual components and their controlled reconstitution in a defined environment. What is clear from these data is that divalent cations, specifically Mg^{2+}, appear to play an important role in the regulation of adenylate cyclase activity.

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REFERENCES

1. Cech, S. Y., W. C. Broaddus, and M. E. Maguire. Adenylate cyclase: the role of magnesium and other divalent cations. *Mol. Cell. Biochem.* **33**:67-92 (1980).
2. Garbers, D. L., and R. A. Johnson. Metal and metal-ATP interactions with brain and cardiac adenylate cyclases. *J. Biol. Chem.* **250**:8449-8456 (1975).
3. Londos, C., and M. S. Preston. Activation of the hepatic adenylate cyclase system by divalent cations: a reassessment. *J. Biol. Chem.* **252**:5957-5961 (1977).
4. Narayanan, N., J.-W. Wei, and P. V. Sulakhe. Differences in the cation sensitivity of adenylate cyclase from heart and skeletal muscle: modification by guanyl nucleotide and isoproterenol. *Arch. Biochem. Biophys.* **197**:18-29 (1979).
5. Bird, S. J., and M. E. Maguire. The agonist-specific effect of magnesium ion on binding by β-adrenergic receptors in S49 lymphoma cells: interaction of GTP and magnesium in adenylate cyclase activation. *J. Biol. Chem.* **253**:8826-8834 (1978).
6. Flatman, P. W. The effect of buffer composition and deoxygenation on the concentration of ionized magnesium inside human red blood cells. *J. Physiol. (Lond.)* **300**:19-30 (1980).
7. Gupta, R. J., and R. D. Moore. ^{31}P NMR studies of intracellular free Mg^{2+} in intact frog skeletal muscle. *J. Biol. Chem.* **255**:3987-3993 (1980).
8. Gupta, R. J., and W. D. Yushok. Noninvasive ^{31}P NMR probes of free Mg^{2+}, MgATP, and MgADP in intact Ehrlich ascites tumor cells. *Proc. Natl. Acad. Sci. U. S. A.* **77**:2487-2491 (1980).
9. Franklin, T. J., and P. A. Twose. Reduction in β-adrenergic response of cultured glioma cells following depletion of intracellular GTP. *Eur. J. Biochem.* **77**:113-117 (1977).
10. Smith, C. M., J. F. Henderson, and H. P. Baer. Effects of GTP on cyclic AMP concentrations in intact Ehrlich ascites tumor cells. *J. Cyclic Nucleotide Res.* **3**:347-354 (1977).
11. Johnson, G. S., and V. R. Mukku. Evidence in intact cells for an involvement of GTP in the activation of adenylate cyclase. *J. Biol. Chem.* **254**:95-100 (1979).
12. Maguire, M. E., and J. J. Erdos. Magnesium but not calcium accumulation is inhibited by β-adrenergic stimulation in S49 lymphoma cells. *J. Biol. Chem.* **253**:6633-6636 (1978).
13. Maguire, M. E., and J. J. Erdos. Inhibition of magnesium uptake by β-adrenergic agonists and prostaglandin E_1 is not mediated by cyclic AMP. *J. Biol. Chem.* **255**:1030-1035 (1980).
14. Erdos, J. J., and M. E. Maguire. Independent desensitization of β-adrenergic receptor-regulated magnesium transport and cyclic AMP accumulation. *Mol. Pharmacol.* **18**:379-383 (1980).
15. Vauquelin, G., S. Y. Cech, C. André, A. D. Strosberg, and M. E. Maguire. Distinctions in *beta*-adrenergic receptor interactions with the guanine nucleotide-magnesium coupling proteins in turkey erythrocyte and S49 lymphoma membranes. *J. Cyclic Nucleotide Res.*, in press (1982).
16. Deleted in proof.
17. Maguire, M. E. Magnesium regulation of the *beta*-receptor-adenylate cyclase complex. II. Sc^{3+} as a Mg^{2+} antagonist. *Mol. Pharmacol.* **22**:274-280 (1982).
18. Ross, E. M., M. E. Maguire, T. W. Sturgill, R. L. Biltonen, and A. G. Gilman. Relationship between the β-adrenergic receptor and adenylate cyclase: studies of ligand binding and enzyme activity in purified membranes of S49 lymphoma cells. *J. Biol. Chem.* **252**:5761-5775 (1977).
19. Salomon, Y., C. Londos, and M. Rodbell. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541-548 (1974).
20. Johnson, G. L., H. R. Kaslow, Z. Farfel, and H. R. Bourne. Genetic analysis of hormone-sensitive adenylate cyclase. *Adv. Cyclic Nucleotide Res.* **13**:1-37 (1980).
21. Williams, L. T., D. Mullikin, and R. J. Lefkowitz. Magnesium dependence of agonist binding to adenylate cyclase-coupled hormone receptors. *J. Biol. Chem.* **253**:2984-2989 (1978).
22. Ross, E. M., A. C. Howlett, K. M. Ferguson, and A. G. Gilman. Reconstitution of hormone-sensitive adenylate cyclase activity with resolved components of the enzyme. *J. Biol. Chem.* **253**:6401-6412 (1978).
23. Bourne, H. R., D. Kaslow, H. R. Kaslow, M. R. Salomon, and V. Licko. Hormone-sensitive adenylate cyclase: mutant phenotype with normally reg-

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ulated *beta*-adrenergic receptors uncoupled from catalytic adenylate cyclase. *Mol. Pharmacol.* **20**:435–441 (1981).
24. Kent, R. S., A. De Lean, and R. J. Lefkowitz. A quantitative analysis of *beta*-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. *Mol. Pharmacol.* **17**:14–23 (1980).
25. Insel, P. A., M. E. Maguire, A. G. Gilman, H. R. Bourne, P. Coffino, and K. Melmon. *Beta*-adrenergic receptors and adenylate cyclase: products of separate genes. *Mol. Pharmacol.* **12**:1062–1069 (1976).
26. Howlett, A. C., P. C. Sternweis, B. A. Macik, P. M. Van Arsdale, and A. G. Gilman. Reconstitution of catecholamine-sensitive adenylate cyclase: association of a regulatory component of the enzyme with membranes containing the catalytic protein and β-adrenergic receptors. *J. Biol. Chem.* **254**:2287–2295 (1979).
27. Iyengar, R., and L. Birnbaumer. Hysteretic activation of adenylyl cyclase. I. Effect of Mg ion on the rate of activation by guanine nucleotides and fluoride. *J. Biol. Chem.* **256**:11036–11041 (1981).
28. Iyengar, R. Hysteretic activation of adenylyl cyclase. II. Mg ion regulation of the activation of the regulatory component as analyzed by reconstitution. *J. Biol. Chem.* **256**:11042–11050 (1981).
29. Sternweis, P. C., J. K. Northup, M. D. Smigel, and A. G. Gilman. The regulatory component of adenylate cyclase: purification and properties. *J. Biol. Chem.* **256**:11517–11526 (1981).
30. Limbird, L. E., A. R. Hickey, and R. J. Lefkowitz. Unique uncoupling of the frog erythrocyte adenylate cyclase system by manganese: loss of hormone and guanine nucleotide sensitive enzyme activities without loss of nucleotide-sensitive, high affinity agonist binding. *J. Biol. Chem.* **254**:2677–2683 (1979).

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