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

II. Sc^{3+} as a Mg^{2+} Antagonist

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SUMMARY

Sc^{3+} bears the same relationship to Mg^{2+} as La^{3+} to Ca^{2+}, a similar ionic radius but increased charge. Therefore, the possibility was investigated that Sc^{3+} would be a Mg^{2+} antagonist at Mg^{2+} sites on the *beta*-adrenergic receptor-adenylate cyclase complex of the murine S49 lymphoma cell. Sc^{3+} is consistently much more potent than La^{3+} in inhibiting adenylate cyclase regardless of the mode of activation. IC_{50} values for Sc^{3+} of 10–30 μM were observed, whereas those for La^{3+} were about 300 μM. However, Sc^{3+} does not block the ability of Mg^{2+} to increase *beta*-receptor affinity for agonist nor alter agonist affinity by itself. Furthermore, Sc^{3+} is a weak inhibitor of the *beta*-receptor-mediated inhibition of Mg^{2+} influx. In *cyc*$^-$ S49 membranes, in which the catalytic subunit of cyclase cannot interact with the nucleotide-coupling protein(s), Sc^{3+} is as potent as in wild-type S49 membranes and again more potent than La^{3+}. Substrate kinetics show that Sc^{3+}, like Mg^{2+}, modulates adenylate cyclase activity by affecting the V_{max} without altering the K_m for substrate. The data suggest that Sc^{3+} is a specific antagonist of Mg^{2+} at the Mg^{2+} site on the catalytic subunit and support the suggestion that there are two distinct sites for Mg^{2+} with different functions, one site on the coupling protein(s) and one on the catalytic subunit. It was also found that an apparent complex of Sc^{3+} and F^-, ScF_4^-, is a potent inhibitor of adenylate cyclase, with an IC_{50} of 3 μM.

INTRODUCTION

The *beta*-receptor-adenylate cyclase complex of the murine S49 cell is modulated by free Mg^{2+}, i.e., regulation of receptor affinity for agonist and activation of adenylate cyclase activity (1, 2). The preceding paper (3) extended these findings to demonstrate that Mn^{2+} ion distinguishes between these two effects, blocking the effect of Mg^{2+} to increase receptor affinity but mimicking the ability of Mg^{2+} to activate cyclase. The Mn^{2+} data in turn suggested that the effects of Mg^{2+} on the receptor-cyclase complex might be mediated by two distinct sites for divalent cation, prompting an investigation of other metal ions that might mimic or antagonize Mg^{2+}.

La^{3+} has long been regarded as a fairly specific Ca^{2+} antagonist (4, 5), presumably because its ionic radius is approximately the same as that of Ca^{2+}, but its net charge is +3 rather than +2. By analogy, Sc^{3+} might antagonize Mg^{2+} since it has approximately the same ionic radius as Mg^{2+}, a net charge of +3, and a chemistry similar to that of La^{3+}. The data in this report suggest that Sc^{3+}, like Mn^{2+}, distinguishes between the effects of Mg^{2+} on the receptor-cyclase complex. Sc^{3+} acts as a Mg^{2+} antagonist with regard to activation of catalysis but has no effect on Mg^{2+} modulation of receptor affinity for agonist. Together, the effects of Mn^{2+} and Sc^{3+} (ref. 3 and this paper) indicate that the receptor-cyclase complex possesses two functionally distinct sites specific for free Mg^{2+}.

METHODS

The methods for assay of adenylate cyclase activity and ligand binding to *beta*-adrenergic receptors were identical with those described previously (1, 3). Unless otherwise stated, the Mg^{2+} concentration was 10 mM and the ATP concentration was 0.5 mM in all adenylate cyclase assays. Mg^{2+} influx measurements were also performed as previously described (6, 7).

Because of the tendency of aqueous solutions of lanthanides to hydrolyze water (8, 9), La^{3+} and Sc^{3+} solutions were always prepared fresh within 20 min of use in an assay. Solutions of the chloride salts of both ions were prepared in 1 mM HCl, and all dilutions were made in HCl. Such solutions were added to assay cocktails 10–15 sec before initiation of the reaction by the addition of

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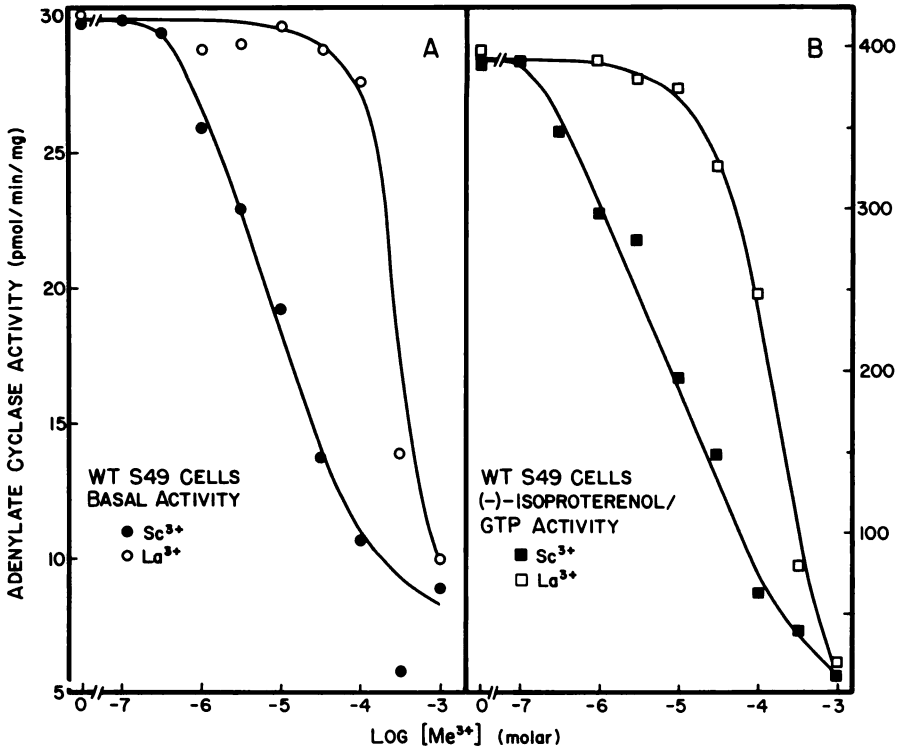

FIG. 1. *Sc^{3+} and La^{3+} inhibition of basal and (−)-isoproterenol/GTP-stimulated adenylate cyclase*

Trivalent cation inhibition was measured as described under Methods. Freshly made solutions of Sc^{3+} and La^{3+} were added 15 sec before initiation of the cyclase assay by addition of membrane protein from wild-type S49 cells. (−)-Isoproterenol and GTP concentrations were 10 μM each.

membrane fractions or intact cells. Assays were also shortened to a maximum of 15 min to minimize hydrolysis and were shown to be linear over this period. In addition, some experiments were performed at pH 7.0, which somewhat diminishes hydrolysis of the free ion to the trihydroxide. These experiments showed results identical with those performed at pH 8.0. In contrast, similar results were not obtained when solutions of Sc^{3+} or La^{3+} were allowed to sit at room temperature at pH 8.0 for periods as short as 1 hr. Thus, freshly made acid solutions and short assay times appear necessary for use of these trivalent cations.

The ScF_4^- complex was formed by adding one volume of 10 mM $ScCl_3$ to one volume of 41 mM NaF and diluting to appropriate concentrations. The formation of ScF_4^- under these conditions should be quantitative with regard to Sc^{3+} according to published constants for the Sc^{3+} and F^- equilibrium (9, 10). At the concentrations of the complex used in the cyclase assays, the excess F^- is micromolar or less and is too low to activate the cyclase by itself, since half-maximal F^- activation in S49 cell membranes occurs at 3 mM F^-.[2] The ScF_4^- solutions must be used immediately because, even though formation of ScF_4^- is essentially quantitative, the minute amount of ScF_3 that forms is extremely insoluble in water, and the precipitation will pull the reaction toward formation of ScF_3; indeed, solutions prepared as described above show significant precipitates within 2 hr of preparation.

[2] M. E. Maguire, unpublished observation.

Although the exact form in aqueous solution of either Sc^{3+} or the Sc-F complex that inhibits adenylate cyclase is not known, published equilibrium constants (8–10) support the suggestion of free Sc^{3+} and ScF_4^-; in any case, the inhibition appears stable over the course of the reaction, since reaction rates are linear for at least 15 min and the rate in the presence of Sc^{3+} or the Sc-F complex extrapolates back through the zero time point.

RESULTS

Figure 1 shows the dose-response curves for Sc^{3+} and La^{3+} inhibition of basal and (−)-isoproterenol/GTP-stimulated adenylate cyclase in membranes from wild-type S49 cells. Sc^{3+} inhibits basal activity with an IC_{50} of about 20 μM, whereas La^{3+} inhibits with an IC_{50} of more than 500 μM (Fig. 1A). Approximately the same IC_{50} values are obtained for both Sc^{3+} and La^{3+} when (−)-isoproterenol/GTP-stimulated activity is measured (Fig. 1B). The ability of Sc^{3+} and La^{3+} to inhibit preactivated adenylate cyclase was measured by incubating membranes with Gpp(NH)p[3] for 20 min prior to addition of inhibitor and substrate (Fig. 2). The time-dependent activation by Gpp(NH)p has previously been shown to reach about 90% of maximal activation after a 20-min incubation in S49 cell membranes (11). In Gpp(NH)p-activated membranes, Sc^{3+} and La^{3+} exhibit respective IC_{50} values sim-

[3] The abbreviations used are: Gpp(NH)p, 5′-guanylyl imidodiphosphate; R, receptor; G/F and C, the regulatory and catalytic components of adenylate cyclase, respectively; IC_{50}, cation concentration giving 50% inhibition at stated $[Mg^{2+}]$ and [MgATP].

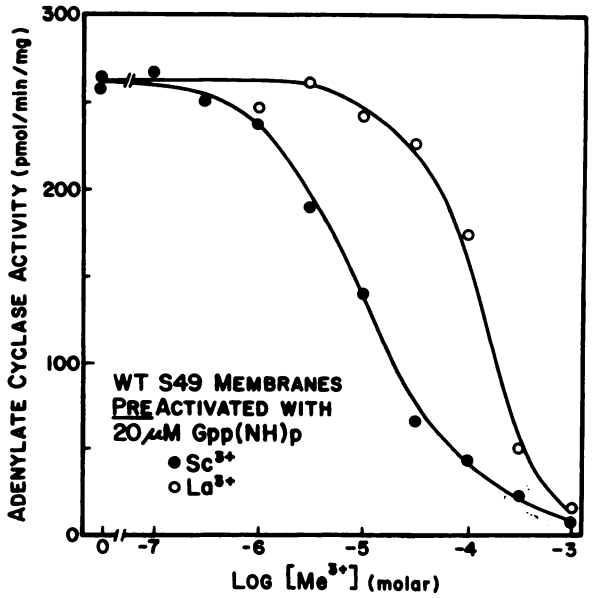

FIG. 2. *Sc^{3+} and La^{3+} inhibition of preactivated adenylate cyclase*

Membranes from wild-type S49 cells were incubated for 20 min at 30° in the presence of 20 μM Gpp(NH)p in the complete adenylate cyclase assay mixture minus trivalent cation and minus [α-^{32}P]ATP. The final volume of the preincubation medium was 50 μl, and therefore the concentrations of reagents in the preincubation were twice the final concentrations (see Methods and ref. 1). At t = 20 min the assay was initiated by the addition (within 10 sec) of trivalent cation and [α-^{32}P]ATP to a final volume of 100 μl.

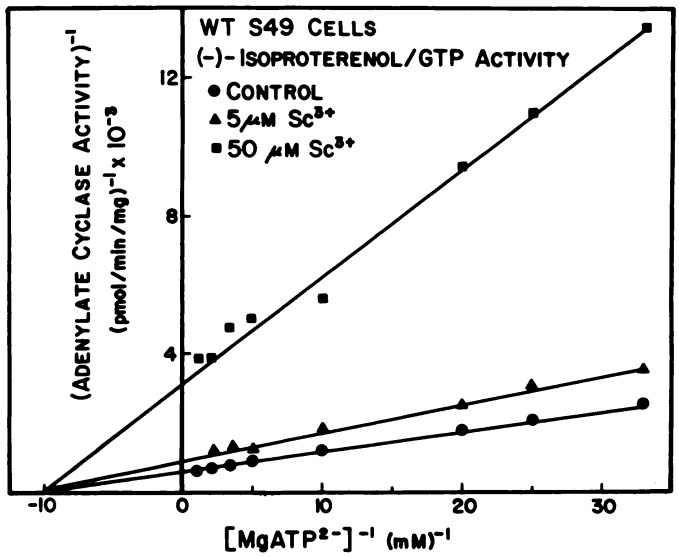

FIG. 3. *Effect of Sc^{3+} on $MgATP^{2-}$ kinetics in membranes from wild-type S49 cells*

Substrate kinetic parameters were determined as described in the legend to Table 1 at eight concentrations of $MgATP^{2-}$ in the absence (●), and in the presence of 5 μM (▲) and 50 μM (■) Sc^{3+}. V_{max} and K_m values determined from these data are listed in Table 1.

ilar to those shown in Fig. 1 for basal and (−)-isoproterenol/GTP-stimulated activities.

There are potentially two mechanisms by which Sc^{3+} could inhibit. It could be acting as a direct Mg^{2+} antagonist by binding at one of the Mg^{2+} regulatory sites (1–3) or it could act by chelating ATP. In the latter case, the inhibition would then be produced either by formation of a nonproductive substrate complex or simply by the sequestration of a significant amount of total ATP. The affinity of ATP for Sc^{3+} and La^{3+} is essentially identical,[4] 0.3 μM for $LaATP^-$ and 0.4 μM for $ScATP^-$. Sequestration should therefore be identical for La^{3+} and Sc^{3+} and thus cannot explain the difference in their dose-response curves. Figure 3 and Table 1 show the results of an experiment to determine whether $ScATP^-$ and $LaATP^-$ are competitive substrate inhibitors. At 5 and 50 μM added Sc^{3+}, there is no effect on the K_m for $MgATP^{2-}$, an indication that $ScATP^-$ is not a competitive substrate inhibitor. Conversely, the dose-dependent decrease in the V_{max} suggests that free Sc^{3+} itself inhibits non-competitively (Fig. 3). These results are not consistent with the possibility that the potent Sc^{3+} inhibition shown in Figs. 1 and 2 is the result of $ScATP^-$ formation but rather indicate a direct effect of free Sc^{3+}, presumably at Mg^{2+} sites. The data with La^{3+} (Table 1) also suggest, at low concentrations, a primary effect on the V_{max} rather than the K_m. At higher La^{3+} concentrations (data not shown) a marked increase in apparent K_m occurs, suggesting that LaATP is either a nonproductive substrate or that La^{3+} sequesters sufficient ATP to reduce substrate concentration.

[4] W. W. Cleland, personal communication.

The ability of Sc^{3+} to alter Mg^{2+} modulation of receptor affinity for agonist was determined next. Figure 4 shows that Sc^{3+} does not block the ability of Mg^{2+} to increase affinity for agonist. Furthermore, Sc^{3+} in the absence of Mg^{2+} has no effect on agonist affinity. These results indicate that Sc^{3+} neither mimics nor blocks Mg^{2+} at the metal site that controls receptor affinity, a site associated with G/F (3, 11).

A second Mg^{2+} site is apparently associated with C and can be tested with the *cyc*$^-$ variant of S49 cells. These results are shown in Fig. 5. At pH 8.0 (Fig. 5B), Sc^{3+} inhibits cyclase activity in *cyc*$^-$ with a potency slightly less than that seen in wild-type membranes and is again much more potent than La^{3+}. One possibility for this slight difference in Sc^{3+} potency in wild-type versus *cyc*$^-$

TABLE 1

Substrate kinetics in membranes from wild-type S49 cells: effect of Sc^{3+} and La^{3+}

Substrate kinetics for (−)-isoproterenol/GTP-activated adenylate cyclase in membranes from wild-type S49 cells were determined as previously described (1) with seven or eight concentrations of $MgATP^{2-}$ from 30 μM to 1 mM with a constant excess of 10 mM Mg^{2+}. V_{max} and K_m values were determined from double-reciprocal plots of activity versus [$MgATP^{2-}$]. The data shown are for a single experiment. Similar data were obtained in a second experiment using a different membrane stock, although the absolute V_{max} and K_m differed slightly.

Condition	V_{max}	K_m	r^2
	pmoles/min/mg	μM	
Experiment 1			
Control	1600	95	0.999
+ 5 μM Sc^{3+}	1060	90	0.998
+50 μM Sc^{3+}	320	101	0.992
Experiment 2			
Control	1705	88	0.999
+ 5 μM La^{3+}	1580	87	0.998
+50 μM La^{3+}	1310	83	0.999

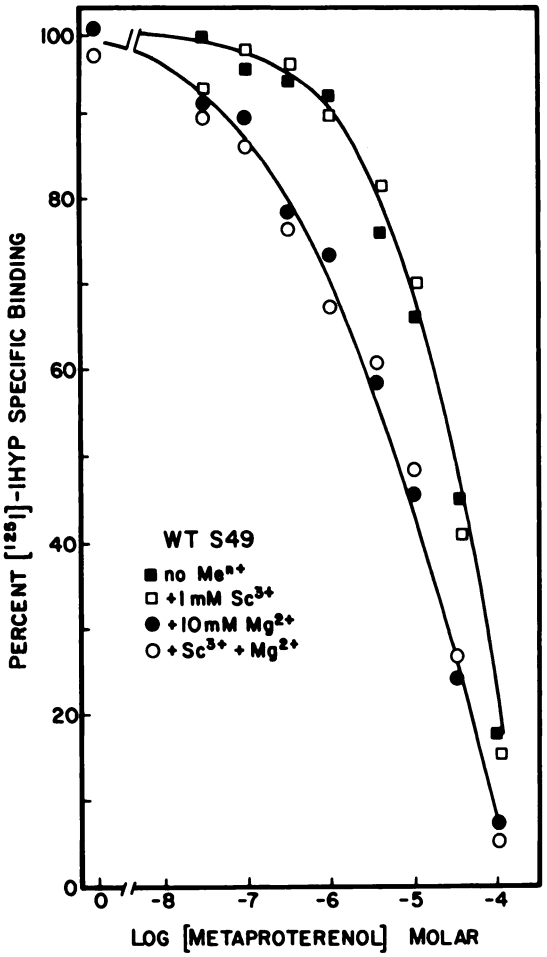

FIG. 4. *Inability of Sc^{3+} to block Mg^{2+} modulation of receptor affinity for agonist*

(±)-Metaproterenol competition for [^{125}I]iodohydroxybenzylpindolol binding in the presence of 10 mM Mg^{2+} and in the absence (●) or presence (○) of 3 mM Sc^{3+}. With (±)-metaproterenol in the complete absence of Mg^{2+} (■), 3 mM Sc^{3+} has no effect on agonist affinity (□). Similar data were obtained using (−)-isoproterenol with or without 1 mM Sc^{3+} (data not shown). The maximal specific binding in the experiment shown was 1115 ± 45 cpm and was 83% of total binding in the experiment shown. The data points represent the average of triplicates whose average variation was 4%.

membranes is the longer assay period of 30 min in *cyc*$^-$ necessitated by its low activity. The longer assay time would allow for more hydrolysis by Sc^{3+}, resulting in a lower effective concentration. At pH 7.0, hydrolysis by Sc^{3+} should be somewhat slower (8, 9). Figure 5A shows that the potency of Sc^{3+} is slightly higher at pH 7.0 than at pH 8.0 (Fig. 5B) and similar to that observed in wild-type cells. The IC_{50} for La^{3+} is not significantly altered by the decrease in pH. It should be noted that *cyc*$^-$ requires $MnATP^{2-}$ as substrate for adenylate cyclase rather than $MgATP^{2-}$. Thus, in the experiments shown, 2.0 mM Mn^{2+} was substituted for the 10 mM Mg^{2+} normally present. Control experiments using membranes from wild-type S49 cells showed that this use of Mn^{2+} for Mg^{2+} did not detectably alter the Sc^{3+} dose-response curve as shown in Fig. 1 (data not shown).

Mg^{2+} but not Ca^{2+} flux in S49 cells is specifically inhibited by the *beta*-adrenergic receptor-adenylate cyclase system (6, 7). Since Sc^{3+} blocks at least one function of the receptor-cyclase complex that is mediated by Mg^{2+}, Sc^{3+} and La^{3+} were tested as inhibitors of Mg^{2+} influx using $^{28}Mg^{2+}$. Figure 6 shows that, in contrast to the potent Sc^{3+} inhibition of adenylate cyclase, La^{3+} is slightly more potent for inhibition of influx. However, neither ion is a particularly effective inhibitor of Mg^{2+} influx; indeed, what inhibition is seen may be due in part to aggregation of intact cells or direct cell toxicity.

The ability of Sc^{3+} to inhibit fluoride-stimulated adenylate cyclase activity was also tested. The results shown in Fig. 7 indicate that Sc^{3+} again is apparently more potent than La^{3+} and superficially resemble those obtained with basal and Gpp(NH)p- and (−)-isoproterenol/GTP-stimulated activities; however, there are problems associated with the combination of Sc^{3+} and F^- related to the aqueous chemistry of Sc^{3+}. According to literature data, Sc^{3+} interacts with F^- to form a series of stable coordination complexes, i.e., ScF^{2+}, ScF_2^+, ScF_3, and ScF_4^-. The equilibrium constants for these reactions (9, 10) indicate that ScF_4^- is the predominant species (>99.9%) at the concentrations of F^- and Sc^{3+} used in the assay shown in Fig. 7. Thus, in contrast to the inhibition of basal and hormone-stimulated cyclase activity, scandium inhibition of fluoride-stimulated cyclase is most likely mediated by ScF_4^-. The data in Fig. 8 in which direct inhibition by ScF_4^- was measured support this interpretation. The apparent IC_{50} for ScF_4^- is about 3 μM, significantly greater than the potency of Sc^{3+} alone on basal or (−)-isoproterenol/GTP-stimulated cyclase activity. The value of 3 μM is also greater than the IC_{50} of 20 μM for Sc^{3+} in the presence of excess F^- (Fig. 7). Furthermore, in the presence of 5 μM ScF_4^-, free Sc^{3+} still inhibits residual basal cyclase activity with an apparent IC_{50} of 30 μM, suggesting that the site of action of Sc^{3+} and ScF_4^- may not be the same (data not shown). The lesser potency of La^{3+} (IC_{50} = 1 mM) for inhibition of fluoride-stimulated cyclase is presumably explained by its much lesser tendency to form the soluble LaF_4^- complex (13).

DISCUSSION

The data presented above show that Sc^{3+} is a potent inhibitor of adenylate cyclase activity. Although it is possible that this inhibition by Sc^{3+} is nonspecific in some manner, this seems unlikely since La^{3+}, a chemically similar trivalent ion, is consistently and significantly less potent than Sc^{3+}. Moreover, the ability of Sc^{3+} to inhibit cyclase activity without affecting interaction of the receptor with G/F is not consistent with a general effect either on interaction between proteins or with the structure of the membrane. More plausible mechanisms for Sc^{3+} inhibition include free Sc^{3+} interaction with specific Mg^{2+} sites; competition with $MgATP^{2-}$ via formation of a nonproductive substrate, $ScATP^-$; or simple substrate sequestration (as $ScATP^-$) without direct interaction of $ScATP^-$ at a substrate binding site.

Of these three possibilities, the latter is not reasonable, as shown by the differential effects of Sc^{3+} versus La^{3+}. Since both Sc^{3+} and La^{3+} have similar affinities for ATP, formation of metal-ATP complexes should be similar at any given Sc^{3+} and La^{3+} concentration, thereby reducing available ATP for substrate formation to an identical

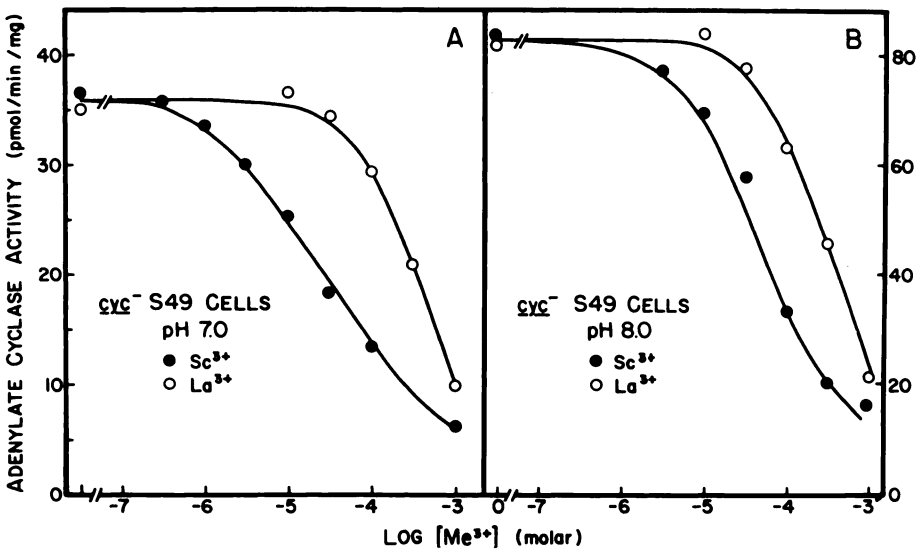

FIG. 5. *Sc^{3+} and La^{3+} inhibition of adenylate cyclase in membranes from cyc⁻ S49 cells*

Trivalent cation inhibition was determined as described under Methods and in the legend to Fig. 1. The pH of the Na-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer was adjusted to 7.0 at 30° in Fig. 5A whereas the pH in Fig. 5B was the normal assay pH of 8.0. The assay time in this experiment was 30 min because of the low activity of *cyc⁻* membranes. Since the *cyc⁻* cyclase requires $MnATP^{2-}$ for substrate, the final concentrations of ATP and divalent cation were 0.5 mM ATP and 2.5 mM Mn^{2+} (see text).

extent and thus giving identical dose-response curves for Sc^{3+} and La^{3+}—a result which is clearly not seen. Furthermore, Sc^{3+} exhibits the same potency for inhibition whether $MnATP^{2-}$ or $MgATP^{2-}$ is used as substrate (data not shown). Since the affinity of Mn^{2+} for ATP is much greater than that of Mg^{2+}, if Sc^{3+} were competing for ATP, its potency should be less in the presence of Mn^{2+} than in the presence of Mg^{2+}. Thus, simple chelation of ATP does not explain these results. Formation of $ScATP^{-}$ and its action as a nonproductive substrate is

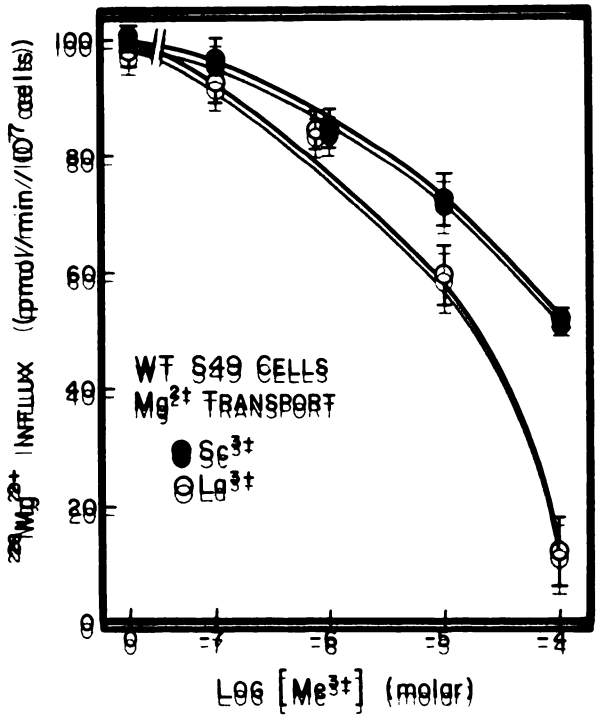

FIG. 6. *Sc^{3+} and La^{3+} inhibition of $^{28}Mg^{2+}$ influx in intact wild-type S49 cells*

Trivalent cations were added in the indicated concentrations to wild-type S49 cells immediately before the addition of tracer $^{28}Mg^{2+}$ to a final total Mg^{2+} concentration of 0.15 mM. Cell density was 6×10^6 cells/ml. Influx of $^{28}Mg^{2+}$ was measured as previously described (5, 6). Maximal influx in the experiment shown was 26 pmoles/min/10^7 cells.

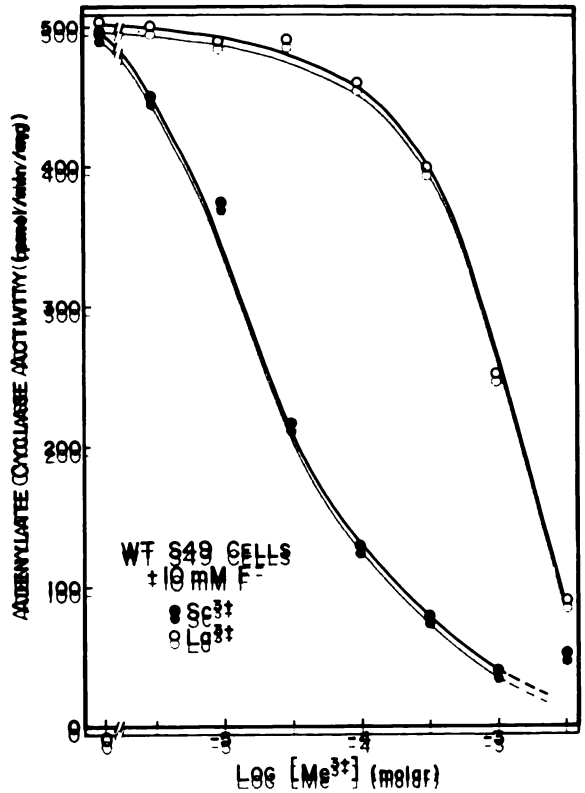

FIG. 7. *Sc^{3+} and La^{3+} inhibition of fluoride-stimulated adenylate cyclase in membranes from wild-type S49 cells*

NaF concentration was 10 mM and trivalent cation was added immediately before initiation of the assay with membrane protein.

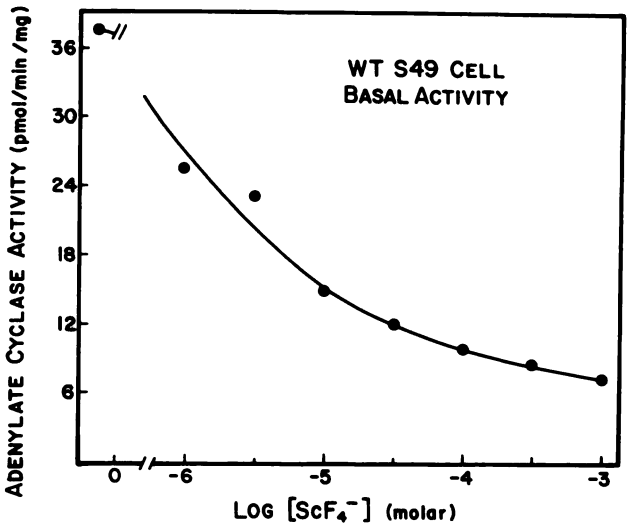

FIG. 8. *ScF_4^- inhibition of basal adenylate cyclase activity in membranes from wild-type S49 cells*

Freshly made ScF_4^- was formed as described under Methods and added to the assay mixture immediately before initiation of the experiment by the addition of membranes. Free F^- ion was 10% of the ScF_4^- concentration.

also not a feasible explanation for Sc^{3+} inhibition, since kinetic analysis (Fig. 3; Table 1) shows that Sc^{3+} has no significant effect on the K_m for $MgATP^{2-}$. Thus, $ScATP^-$ is not a competitive substrate inhibitor at Sc^{3+} concentrations (5 and 50 μM) which significantly inhibit cyclase activity. Therefore, Sc^{3+} appears to inhibit adenylate cyclase activity by interaction at a Mg^{2+}-specific site(s) and preventing Mg^{2+} activation.

In the course of these studies we noted a novel inhibition of adenylate cyclase by an F^- complex of Sc^{3+}, ScF_4^-. This inhibition is not due to sequestration of F^- ion and inhibition of activation per se, since in the experiment shown in Fig. 7, F^- is in great excess of Sc^{3+}. The mechanism of this inhibition is unclear. ScF_4^- might specifically inhibit F^- activation via binding of one fluoride of the ScF_4^- complex to the F^- site on the cyclase complex. However, this seems unlikely since ScF_4^- inhibits basal activity (Fig. 8) even more potently than it does fluoride-stimulated activity.

The most interesting aspect of these results is the suggestion that two independent metal ion sites exist on the receptor-cyclase complex. The results indicate that a Mg^{2+} site on G/F is responsible for modulation of receptor affinity for agonist. Direct support for this conclusion derives from two sources. First, Mg^{2+} increases receptor affinity for agonist in HC-1 cells and in the H21a variant of S49 cells (3). Since the catalytic subunit in HC-1 is absent or nonfunctional (14) and since in H21a cells C cannot interact with G/F (15), this metal site cannot be on C. Likewise, the site is most probably not on the receptor, since Mg^{2+} does not alter receptor affinity for agonist in the *cyc*$^-$ and *unc* variants of S49 cells (1) where R cannot interact with G/F. It is possible that the metal site is on R but requires interaction of R and G/F to exert any effect. Regardless, it is clear that the metal site modulating receptor affinity for agonist is not on C and is most probably on G/F.

The existence of a second metal site on C which is responsible for metal activation of catalysis is supported by the effects of Mn^{2+} and Sc^{3+} on cyclase activity in *cyc*$^-$ cells. Free Mn^{2+} activates C in *cyc*$^-$ membranes (3), and Sc^{3+} specifically inhibits Mn-dependent activity in *cyc*$^-$ membranes (Fig. 5). Both do so with the same affinity as observed in wild-type cells. Furthermore, the effect of Sc^{3+} is specific since it only inhibits catalysis, having no effect at all on receptor affinity for agonist whether in the absence or presence of Mg^{2+} (Fig. 4). Since G/F is absent or at the least nonfunctional in *cyc*$^-$ cells, the site modulating catalytic activity presumably resides on C.

A puzzling aspect of these results has to do with the Mg^{2+} dependence of adenylate cyclase activity in the S49 cell. We have previously described in detail the Mg^{2+} dependence of both receptor binding and adenylate cyclase activation and showed that Mg^{2+} specifically activates adenylate cyclase by increasing the V_{max} without effect on the K_m (1). However, this increase in V_{max} was only seen for basal and GTP- and fluoride-stimulated activities. In the presence of (−)-isoproterenol/GTP, the presumed physiologically relevant state, dependence of the V_{max} on Mg^{2+} was apparently abolished. An alternative explanation (2) is that, in the presence of (−)-isoproterenol/GTP, only micromolar free Mg^{2+} is required for activation, the kinetic experiments being too insensitive to distinguish these possibilities. In other cell systems the effect of hormone plus GTP is to shift the Mg^{2+} dose-response curve for activation to the left, thus requiring lower free Mg^{2+} concentrations for full activation (for review, see ref. 2). However, if the (−)-isoproterenol/GTP-stimulated activity in S49 cells is dependent on extremely low free Mg^{2+}, this ought to be reflected in the potency with which Sc^{3+} inhibits (−)-isoproterenol/GTP-stimulated activity versus its inhibition of basal activity. Since Sc^{3+} inhibits both activities with the same apparent IC_{50}, this suggests (a) that Sc^{3+} is not acting as a Mg^{2+} antagonist, or (b) that the Mg^{2+} dependence of the two forms of cyclase activity is identical, or (c) that an empty Mg^{2+} site is present during (−)-isoproterenol/GTP stimulation that can be occupied by Sc^{3+}. Given the arguments presented above and the differential effects of Sc^{3+} and Mn^{2+} on the various functional forms of adenylate cyclase or receptor, the most likely explanation is the existence of two metal sites. The site on G/F would need to be occupied during hormonal stimulation in order to maintain coupling, whereas the site on C need not be occupied during such stimulation.

Whether two sites exist and whether they function as has been suggested herein is obviously a matter for further experimentation with more purified and defined components. The data strongly suggest the existence of two metal sites and clearly indicate that divalent cations, specifically Mg^{2+}, are important modulatory agents of the receptor-adenylate cyclase complex. Although the data in this and the preceding paper (3) have shown this only for a *beta*-receptor-linked cyclase, the widespread effects of Mg^{2+} on receptor and cyclase activities (1, 2) indicate that Mg^{2+}, like GTP, is an important and ubiquitous modulatory agent of the receptor-cyclase complex regardless of receptor type.

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