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Agonist-Induced Modulation of Inverse Agonist Efficacy at the β_2-Adrenergic Receptor

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SUMMARY

Sustained stimulation of several G protein-coupled receptors is known to lead to a reduction in the signaling efficacy. This phenomenon, named agonist-induced desensitization, has been best studied for the β_2-adrenergic receptor (AR) and is characterized by a decreased efficacy of β-adrenergic agonists to stimulate the adenylyl cyclase activity. Recently, several β-adrenergic ligands were found to inhibit the spontaneous agonist-independent activity of the β_2AR. These compounds, termed inverse agonists, have different inhibitory efficacies, ranging from almost neutral antagonists to full inverse agonists. The current study was undertaken to determine whether, as is the case for agonists, desensitization can affect the efficacies of inverse agonists. Agonist-promoted desensitization of the human β_2AR expressed in Sf9 cells potentiated the inhibitory actions of the inverse agonists, with the extent of the potentiation being inversely proportional to their intrinsic activity. For example, desensitization increased the inhibitory action of the weak inverse agonist labetalol by 29%, whereas inhibition of the spontaneous activity by the strong inverse agonist timolol was not enhanced by the desensitizing stimuli. Interestingly, dichloroisoproterenol acted stochastically as either a weak partial agonist or a weak inverse agonist in control conditions but always behaved as an inverse agonist after desensitization. These data demonstrate that like for agonists, the efficacies of inverse agonists can be modulated by a desensitizing treatment. Also, the data show that the initial state of the receptor can determine whether a ligand behaves as a partial agonist or an inverse agonist.

Recent studies on GPCRs have shown that antagonists can regulate activity in a manner seemingly opposite that of the corresponding agonists (1–5). The measurement of this phenomenon, usually referred to as inverse agonism, requires that the receptor exhibits a detectable level of agonist-independent, spontaneous activity. Although such spontaneous activity can be detected in systems expressing relatively low concentrations of receptors (2–4), it is more easily detectable in overexpression systems, such as the baculovirus/Sf9 cells (2, 5).

Similar to differences among activating ligands, which can be classified as either full or partial agonists, maximal inhibitory activity has been observed to differ among inverse agonists at GPCRs (1, 2, 5, 6). The intrinsic activity of an agonist refers to its propensity to increase the activity of a receptor (7); inverse agonists analogously are said to have negative intrinsic activity (1). The maximal effect of a partial agonist observed experimentally is derived from both the intrinsic activity of the ligand and the sensitivity of the preparation being studied; partial agonists thus may fully activate a receptor response in a highly sensitive system while having relatively little effect in an insensitive one (7). The same principles presumably apply to full and partial inverse agonism, but the aforementioned dependence on spontaneous receptor activity implies a further level of complexity. The net effect of an inverse agonist in a particular system thus may reflect at least three factors: the sensitivity of the system to inverse agonism, the level of spontaneous receptor activity, and the negative intrinsic activity of the ligand.

Recent reports in the literature seem to support the general idea that receptors isomerize rapidly between an active state (R*) and an inactive state (R) and that agonists produce their effects by preferentially binding to and thereby increasing the proportion of the active state, whereas inverse agonists analogously favor the inactive state (8, 9). In terms of such models, the intrinsic activity of a ligand reflects the difference in its affinities for the two states. Thus, compared with full agonists and full inverse agonists, partial agonists and partial inverse agonists, respectively, are thought to be less selective for the active and inactive states of the receptor.

Sustained treatment of the β_2AR-expressing cells with agonist is known to alter signaling efficacy of agonists. This phenomenon, referred to as agonist-promoted desensitiza-

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ABBREVIATIONS: AR, adrenergic receptor; DCI, dichloroisoproterenol; GPCR, G protein-coupled receptor.

tion, is characterized by decreases in agonist potency and efficacy (10, 11). Rapid phosphorylation of the receptor by the βAR kinase and the cAMP-dependent protein kinase is believed to play a central role in this desensitization process (10). The influence of such a regulatory process on the efficacy of inverse agonists has never been investigated. This study represents a first attempt to determine whether agonist-promoted desensitization can alter the efficacy of ligands that inhibit the spontaneous activity of the β_2AR.

Materials and Methods

Culture and infection of Sf9 cells. Sf9 cells were cultured at 27° in Grace's supplemented insect medium (GIBCO, Grand Island, NY) containing 10% fetal bovine serum, 0.1% Pluronic F-68 (GIBCO), 50 μg/ml gentamycin sulfate, and 2.5 μg/ml fungizone. Cells (4×10^6 cells/ml) were infected for 48 hr with a recombinant baculovirus encoding the *c-myc*-tagged human β_2AR, as described previously (2, 12). Cells were treated with either 1 μM isoproterenol or vehicle for the final 30 min of infection.

Preparation of membranes. Washed membranes from Sf9 cells were prepared essentially as described previously (2). Infected cells were rinsed twice with 2 volumes of cold phosphate-buffered saline and then lysed on ice with a Polytron homogenizer (two 5-sec bursts) in 15 ml of buffer containing 5 mM Tris·HCl, pH 7.4, 2 mM EDTA, and protease inhibitors (5 μg/ml leupeptin, 5 μg/ml soybean trypsin inhibitor, and 10 μg/ml benzamidine). Lysates were centrifuged at 500 × *g* for 5 min at 4°, and the resulting supernatant was centrifuged at 45,000 × *g* for 20 min at 4°. The resulting pellet was washed twice in 10 ml of the same buffer, and the final pellet was resuspended in a buffer containing 75 mM Tris·HCl, pH 7.4, 12.5 mM MgCl2, 2 mM EDTA, and protease inhibitors as indicated above for the lysis buffer. Protein content was determined according to the method of Bradford using bovine serum albumin as the control (13).

Assay of adenylyl cyclase activity. Membrane adenylyl cyclase activity was measured as described previously (2, 12). Twenty microliters of membrane suspension (5–10 μg of protein) was added to a reaction cocktail to yield 50-μl samples containing 0.12 mM ATP, 1–2 × 10^6 cpm of [α-^{32}P]ATP, 0.10 mM cAMP, 53 μM GTP, 2.7 mM phospho(enol)pyruvate, 1.0 IU of myokinase, and 0.2 IU of pyruvate kinase. Incubations were carried out for 15 min at 37° and terminated by the addition of an ice-cold solution containing 0.4 mM ATP, 0.3 mM cAMP, and 20,000 cpm of [^{3}H]cAMP. cAMP was separated by sequential chromatography on columns containing Dowex gel and aluminum oxide (14).

Determination of β_2AR density in Sf9 cell membranes. β_2AR densities in membranes prepared as described above were estimated using the specific radioligand ^{125}I-cyanopindolol at a near-saturating concentration (≥0.3 nM; K_d = 33 pM), as described previously (2). Specific binding was taken as the difference between total and nonspecific ^{125}I-cyanopindolol binding, the latter being defined as the radioactive signal in the presence of an excess of propranolol (10 μM).

Analysis of results. Dose-response data were analyzed using a four-parameter logistic equation analogous to the Hill equation (ALLFIT; courtesy of Dr. A. De Lean, Université de Montréal, Montréal, Canada) with the slope factor set to 1, as described previously (2).

Results

To assess the influence of agonist-promoted desensitization on the efficacy of a series of inverse agonists, β_2AR-expressing Sf9 cells were pretreated or not with the β-adrenergic agonist isoproterenol (1 μM) for 30 min. Membranes were then prepared, and the effects of various adrenergic ligands on the adenylyl cyclase activity were assessed. As can be seen in Fig. 1A, pretreatment of the cells with the agonist resulted in a decrease in the maximal stimulation of cAMP production by isoproterenol. This desensitization of the isoproterenol-stimulated adenylyl cyclase activity was accompanied by little or no change in basal or NaF- or forskolin-stimulated enzymatic activity (data not shown). This desensitization pattern is consistent with the phenomenon generally referred to as receptor-specific or homologous desensitization. Also demonstrated in Fig. 1A is the ability of a series of β-adrenergic inverse agonists to inhibit the basal adenylyl cyclase activity. As previously reported (2, 15), the

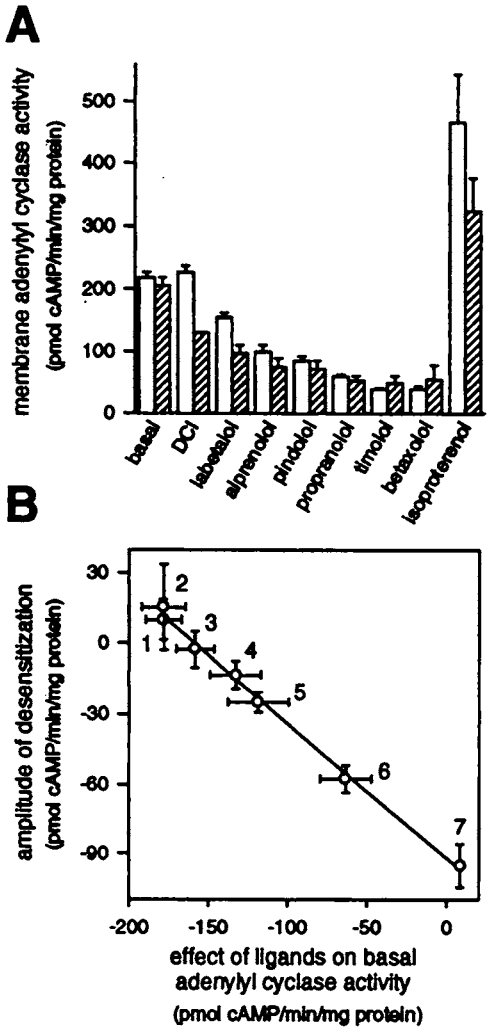

Fig. 1. Effect of desensitizing treatment on β_2AR responsiveness. Sf9 cells were infected 48 hr with recombinant β_2AR baculovirus. For the final 30 min of infection, cells were treated with either vehicle (□) or 1 μM isoproterenol (■). The production of cAMP in membranes was measured in duplicate in two independent experiments in the absence of ligand and in the presence of maximally effective concentrations of the β-adrenergic ligands indicated (DCI and betaxolol, 100 μM; others, 10 μM). A, cAMP production in membranes from control and isoproterenol-treated cells. B, For each inhibitory ligand, the difference obtained in A between cAMP productions in control and desensitized membranes is plotted versus the effect of the ligand on basal adenylyl cyclase activity in control conditions (r^2 = 0.996); the ligands corresponding to the points shown are betaxolol (*1*), timolol (*2*), propranolol (*3*), pindolol (*4*), alprenolol (*5*), labetalol (*6*), and DCI (*7*). β_2AR density was 34 ± 4 pmol of receptor/mg of protein in membranes from control cells and 29 ± 7 pmol of receptor/mg of protein in membranes from isoproterenol-treated cells. Data are mean ± standard deviation.

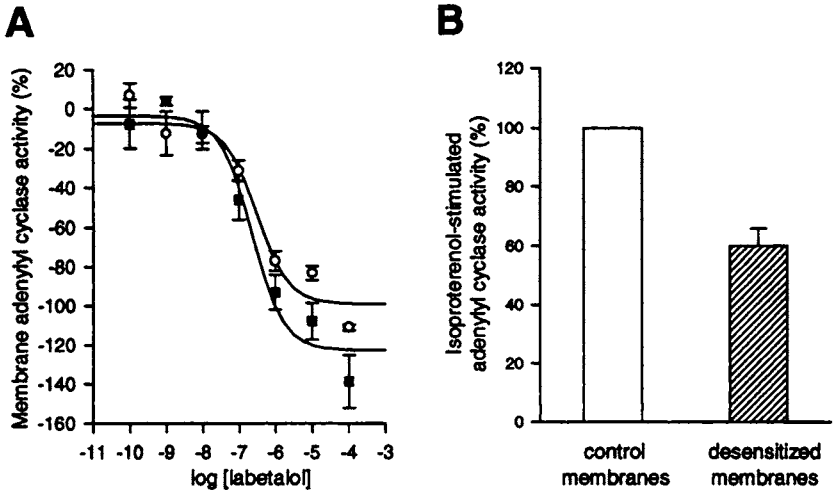

Fig. 2. Effect of desensitizing treatment on β_2AR regulation by labetalol. Cells were treated as described in the legend to Fig. 1, and membrane adenylyl cyclase activity was measured at the indicated concentrations of labetalol in control (○) and desensitized (■) preparations. A, Mean dose-response curves (from eight experiments) for labetalol inhibition of adenylyl cyclase activity in control and desensitized membranes. Data were scaled with the maximal inhibition in control membranes set as 100% for each set of data. B, Stimulation by isoproterenol (10^{-5} M) of adenylyl cyclase activity in control (□) and desensitized (▨) preparations that were used in the experiments represented in A. Data were scaled with the isoproterenol stimulation in control membranes set as 100%. Data are mean ± standard error.

negative intrinsic activity varies considerably among the various ligands tested. In this set of experiments, betaxolol, timolol, and propranolol were found to be the most efficacious inverse agonists. The inhibition of spontaneous β_2AR activity by these inverse agonists was only moderately affected by the desensitization protocol. Thus, even though the receptor was less able to be stimulated by the agonist tested (isoproterenol), overall it remained spontaneously active, and the inhibition of that activity by inverse agonists persisted.

In contrast with what was observed for the most efficacious inverse agonists, desensitization clearly affected the efficacy of the weakest ones, with their inhibitory properties being enhanced by desensitization. This effect is particularly evident for labetalol and DCI. In membranes derived from control cells, labetalol inhibited the basal (i.e., ligand-independent) adenylyl cyclase activity by 29%, whereas this inhibition reached 53% in membranes from desensitized cells. DCI, which was marginally stimulatory in control membranes, inhibited the spontaneous activity by 37% after isoproterenol-induced desensitization. The desensitizing treatment also seemed to modestly enhance the inhibitory effects of the ligands with intermediate inverse efficacy such as alprenolol and pindolol. Interestingly, the effect of desensitization on the inhibitory properties of β_2AR ligands was inversely proportional to their negative intrinsic activity in control membranes, as illustrated by the negative correlation shown in Fig. 1B. Because desensitization had no effect on receptor density (not shown), the increases in inhibition seem to reflect a change in the responsiveness of the receptor *per se*.

Additional experiments were carried out to characterize in more detail the effects of β_2AR desensitization on receptor responsiveness to inverse agonists. Three compounds were selected: labetalol and DCI, the inverse efficacies of which seemed to be most influenced by desensitization, and timolol, as an example of a compound not influenced by the desensitization protocol. In each set of experiments, cells were treated or not with isoproterenol (1 μM) for 30 min, and membranes were prepared and assayed for adenylyl cyclase activity in the presence of increasing concentrations of β_2AR ligands. In each experiment, the extent of agonist desensitization reached was assessed by measuring the maximal (1×10^{-5} M) isoproterenol-stimulated cAMP production in both control and desensitized membranes.

The effects of isoproterenol pretreatment on the inverse agonist properties of labetalol are presented in Fig. 2. The agonist pretreatment led to a 40 ± 6% desensitization of the maximal isoproterenol-stimulated adenylyl cyclase activity (Fig. 2B). As shown in Fig. 2A, the inhibition of membrane cAMP production by labetalol was dose dependent in both control and desensitized membranes, but desensitization clearly potentiated the inhibitory effects of labetalol over a

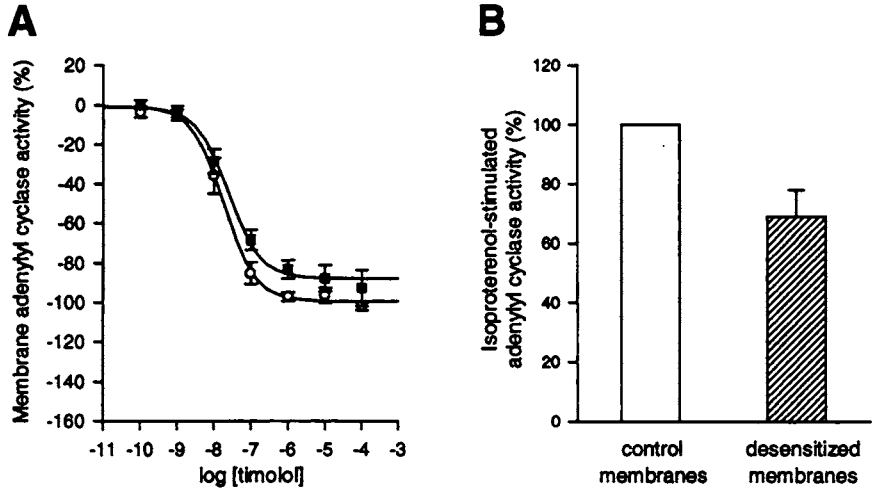

Fig. 3. Effect of desensitizing treatment on β_2AR regulation by timolol. Cells were treated as described in the legend to Fig. 1, and membrane adenylyl cyclase activity was measured at the indicated concentrations of timolol in control (○) and desensitized (■) preparations. A, Mean dose-response curves (from four experiments) for timolol inhibition of adenylyl cyclase activity in control and desensitized membranes. Data were scaled with the maximal inhibition in control membranes set as 100% for each set of data. B, Stimulation by isoproterenol (10^{-5} M) of adenylyl cyclase activity in control (□) and desensitized (▨) preparations used in experiments represented in panel A. Data were scaled with the isoproterenol stimulation in control membranes set as 100%. Data are mean ± standard error.

wide range of concentrations. The figure shows a summary of the inhibitory effects of labetalol in control and desensitized membranes for eight experiments in which the maximal inhibitory effect of labetalol in control membranes was normalized to 100%. Overall, the desensitization promoted a 29 ± 10% increase in the maximal inverse efficacy of labetalol.

In contrast to what was observed for the relatively weak inverse agonist labetalol, desensitization had only marginal effects on the efficacy of the strong inverse agonist timolol. Indeed, as shown in Fig. 3, the desensitization protocol, which lead to a reduction of 31 ± 9% of the isoproterenol-stimulated adenylyl cyclase activity (Fig. 3B), did not appreciably affect the inhibitory dose response of timolol (Fig. 3A). If anything, desensitization tended to marginally (13 ± 6%) decrease the maximal inhibitory response.

In the preliminary set of experiments (see Fig. 1A), DCI was found to be slightly stimulatory in control membranes. As further experiments were carried out to better characterize the effect of β_2AR desensitization on DCI responsiveness, high interexperimental variability of DCI properties in control membranes was observed. DCI could be either moderately stimulatory or inhibitory. Interestingly, very rarely could it be considered a neutral antagonist. Because partial agonists (or inverse agonists) may be more sensitive than full agonists (or inverse agonists) to variations in receptor density, the possible relationship between membrane receptor number and DCI activity was investigated. A comparison over several series of experiments between β_2AR density and the effect of DCI did not reveal any obvious correlation between these two parameters. Fig. 4 presents an histogram of the distribution of DCI efficacy in 44 (control) and 21 (desensitized) independent experiments. In control membranes (Fig. 4A), the dispersion of the efficacies could best be fitted to a bimodal distribution with DCI behaving either as a partial agonist (with a median of 17%) or a partial inverse agonist (with a median of −24%). In desensitized membranes, however, a unimodal distribution was obtained with DCI always behaving as an inverse agonist (median of −37%).

Because DCI can be classified as either a partial agonist or a partial inverse agonist in control membranes, the effect of desensitizing treatment on its responsiveness was evaluated separately in each case. Fig. 5A illustrates the effect of desensitization on DCI efficacy when it behaved as a partial agonist in control membranes. Desensitization had a dramatic effect on the drug efficacy. Indeed, in each experiment, DCI invariably behaved as an inverse agonist in membranes from desensitized cells. The propensity of the partial agonist DCI to become an inverse agonist after desensitization could not be generalized to other agonists because in the same series of experiments, the "full" agonist isoproterenol remained an agonist in desensitized membranes, although with a 40 ± 6% less-stimulatory effect (Fig. 5B).

When, as shown in Fig. 6, DCI was a moderate inverse agonist in control membranes, desensitization potentiated its inhibitory properties over a wide range of concentrations, much like what was observed for labetalol. Therefore, whether DCI behaved as a partial agonist or an inverse agonist in membranes derived from control cells, desensitization always increased the inhibitory properties of this dual ligand.

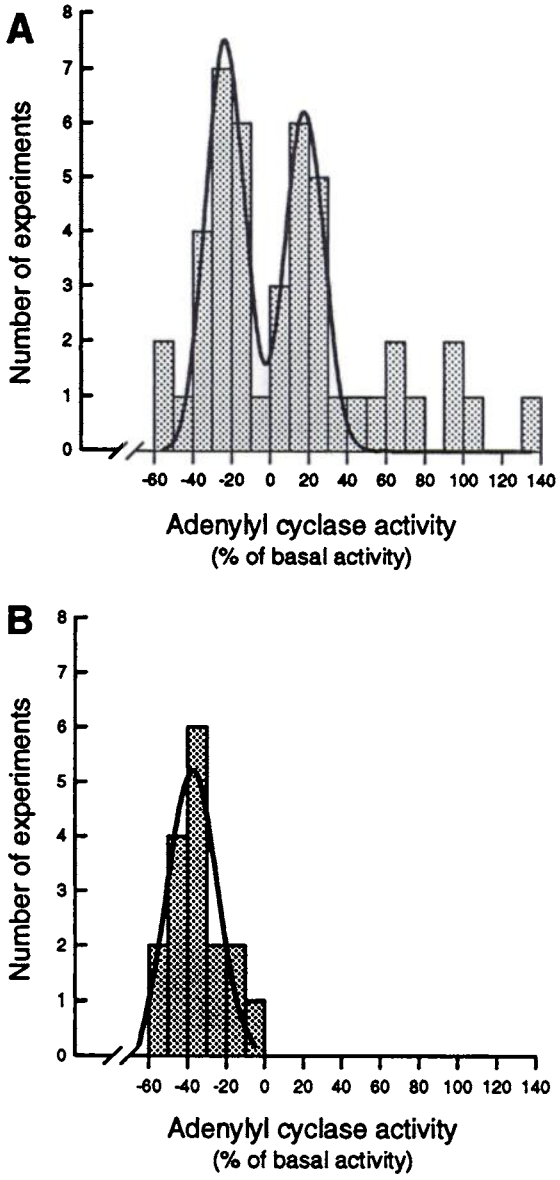

Fig. 4. Dual properties of DCI at the β_2AR. Cells were treated as described in the legend to Fig. 1, and the effect of a maximal concentration of DCI (10^{-4} M) on basal adenylyl cyclase activity was evaluated in duplicate in control and desensitized membranes. A, Histogram of distribution of maximal DCI efficacy in control preparations (44 experiments). B, Histogram of distribution of maximal DCI efficacy in desensitized preparations (21 experiments). Gaussian fits were obtained using SigmaPlot 4.17 (Jandel Scientific, San Rafael, CA).

Discussion

The results of the current study show that agonist-promoted desensitization, which decreases the responsiveness of β_2AR to agonist stimulation, also influences inverse agonism. In this study, a desensitizing treatment was shown to have a rather selective effect on weakly inhibitory ligands such as labetalol, with relatively little effect on highly efficacious inverse agonists like timolol. Perhaps more dramatically, the results clearly show that a ligand such as DCI, which can behave as a partial agonist in control membranes, becomes an inverse agonist after desensitization.

The efficacy of some β_2-adrenergic ligands has been previously shown to differ between intact β_2AR-expressing Sf9

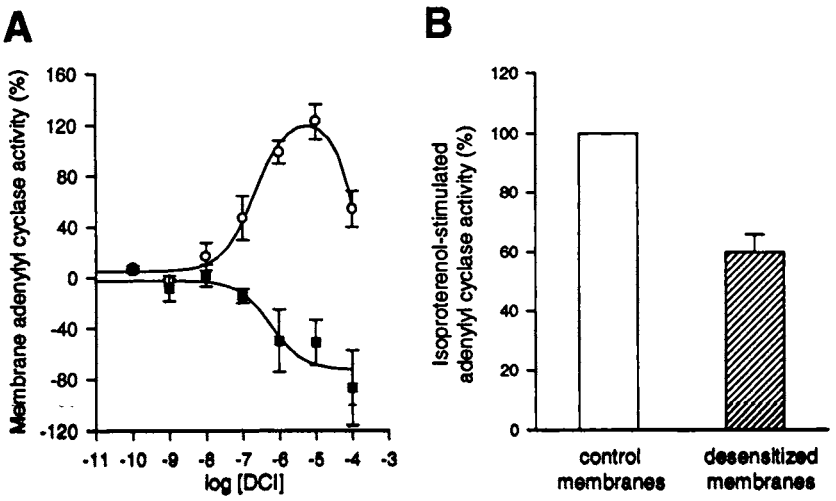

Fig. 5. Effect of desensitizing treatment on agonist properties of DCI. Cells were treated as described previously, and membrane adenylyl cyclase activity was measured at the indicated concentrations of DCI in control (○) and desensitized (■) preparations. A, Mean dose-response curves from five experiments in control and desensitized membranes. Data were scaled with the maximal inhibition in control membranes set as 100% for each set of data. B, Isoproterenol stimulation (10^{-5} M) of adenylyl cyclase activity in control (□) and desensitized (▨) preparations used in experiments represented in A. The stimulation obtained in control membranes was set at 100%. Data are mean ± standard error.

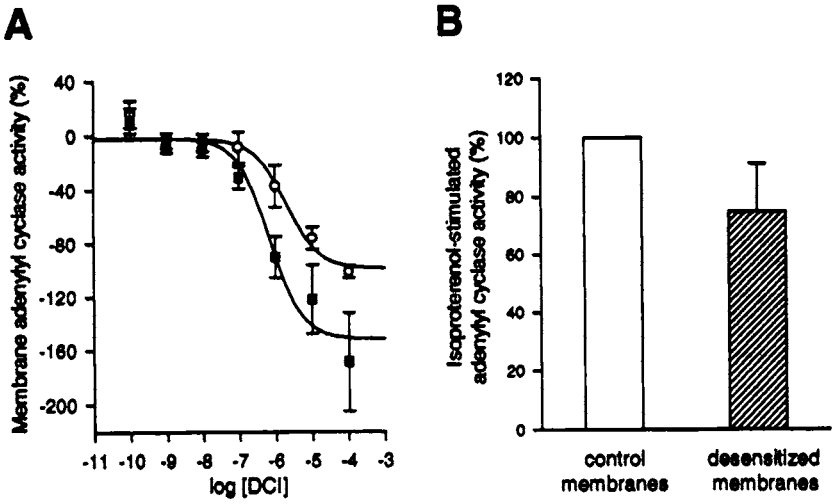

Fig. 6. Effect of desensitization on inverse agonist properties of DCI. Cells were treated as described previously, and membrane adenylyl cyclase activity was measured at the indicated concentrations of DCI in control (○) and desensitized (■) preparations. A, Mean dose-response curves from five experiments in control and desensitized membranes. Data were scaled with the maximal inhibition in control membranes set as 100% for each set of data. B, Isoproterenol stimulation (10^{-5} M) of adenylyl cyclase activity in control (□) and desensitized (■) preparations used in experiments shown in A. The stimulation obtained in control membranes was set at 100%. Data are mean ± standard error.

cells and membranes derived from those cells (2). The present results additionally demonstrate that ligand activity in Sf9 membranes can be modulated by agonist pretreatment. Labetalol stimulates adenylyl cyclase activity in whole cells but inhibits it in membrane assays. DCI acts as a partial agonist in whole cells and behaves as either a partial agonist or a partial inverse agonist in membranes prepared according to the present protocol. After desensitization, the inhibitory properties of both DCI and labetalol become manifest. Taken together, these results suggest that some ligands may have dual stimulatory and inhibitory activities, with the predominating effect depending at least in part on the initial conditions of the system under investigation. Interestingly, under control conditions, DCI seems to act as a partial agonist with a bell-shaped dose-response curve, with the maximal agonistic effect being observed at 10 μM. Similar bell-shaped curves were reported for nonpeptide angiotensin partial agonists and were interpreted as reflecting both agonistic and antagonistic properties of the ligands at different concentrations (16). Whether this bimodal action is related to the dual stimulatory and inhibitory actions of DCI in control and desensitized membranes remains to be investigated.

Regardless of the experimental method used to measure β_2AR modulation in Sf9 cell preparations, the rank order of efficacy of the ligands tested in this study and a previous study (2) seems to be invariant. Furthermore, the same rank order is observed with mammals *in vivo* as well as with membranes prepared from mammalian cells heterogously expressing the human β_2AR (2). Interestingly, this consistent sequence seems to be interrupted only by the variable "null point" of ligand-independent β_2AR activity. For example, under all conditions tested, the observed activity of the receptor in the presence of DCI is greater than that in the presence of pindolol; in contrast, "basal" receptor activity has been observed under various conditions to be less than, equal to, or more than receptor activity in the presence of these ligands. Consequently, each can exhibit partial agonism, "neutral" antagonism, or partial inverse agonism, respectively, depending on the responsiveness of the system under investigation (2, 15, 17–20).

It is generally accepted that the regulation of receptor activity by agonists is sensitive to environmental factors that can lead to physical changes in the receptor itself or its associated proteins. Agonist treatment, which affects β_2AR responsiveness to specific ligands in the current study, has been shown previously to modify receptor structure. Indeed, desensitization is accompanied by increases in β_2AR phosphorylation (10) and possibly by changes in palmitoylation status (12, 21). Such structural alterations may influence the tendency of a receptor to assume active (R*) or inactive (R) states and thereby affect its responsiveness. Also, the efficacy

of R* to activate its cognate G protein may be reduced after desensitization.

In previously described schemes for desensitization, several authors have suggested that the waning of agonist stimulation over time, in preparations containing intact cells, reflects a reversible, agonist-promoted conversion of activatable receptor (R) to a relatively unresponsive state (Rd) (22, 23). Biochemical evidence implies that such interconversion at the β_2AR is accompanied by post-translational modifications such as phosphorylation and dephosphorylation (24, 25). The membrane preparations studied in the current study thus may be viewed as having two subpopulations of β_2AR that do not interconvert appreciably on the time scale of effector assays carried out in cell-free systems. Because the proportional amounts of R and Rd would be expected to differ between membranes from control and agonist-treated cells, differences in receptor activity potentially could be attributed to changes in the balance of these metastable isoforms of the receptor. Also, the variability of the effect of DCI in control membranes might reflect interexperimental differences in the proportion of desensitized receptors already present under control conditions in these membranes. This is supported by the fact that the extent of isoproterenol-promoted desensitization that could be observed was smaller in experiments in which DCI behaved as an inverse agonist in control membranes (compare Figs. 6B and 5B).

As mentioned previously, it is now generally accepted that the nondesensitized receptor isomerizes rapidly between an inactive (R) and an active (R*) conformation, which accounts for spontaneous receptor activity and the inhibitory activities of some specific GPCR ligands. In the current study, both spontaneous receptor activity and inverse agonism continued to be observed in membranes from desensitized cells. Similarly, inverse agonism has been detected in preparations in which GPCRs display little or no response to agonists (5, 26). Furthermore, the constitutively active mutant CAM-β_2AR is reported to be constitutively desensitized and phosphorylated while also being sensitive to inverse agonists (27). These observations raise the question of the existence of an active conformation for the desensitized form of the receptor, which would be designated Rd*. If both R* and Rd* entities exist, then each could contribute to the observed ligand-independent and -regulated signals. Thus, differences in the responsiveness of control and desensitized membranes to β_2AR ligands tested in this study may reflect differences in the selectivity of these compounds for the active versus the inactive state of each isoform of the receptor. To test whether such a model could provide a mechanistic basis to explain the data obtained in the current study, simulations using the two-state model [e.g., Leff (9)] were carried out. In such a model, the effects of agonists and inverse agonists are the result of their preferential binding to the active and inactive states of the receptor, respectively; neutral ligands, in contrast, bind to both forms with equal affinity. It follows that the intrinsic efficacy of a given ligand reflects the ratio of its affinity for R* versus R. This parameter can be defined as $\alpha = K_L/K_L$. If both control and desensitized isoforms are capable of assuming an active conformation, then decreased agonist responsiveness after desensitization can be rationalized as a decrease in relative affinity for the active form of the receptor. Therefore, desensitization can be simulated by increasing the value given to the parameter α. Fig. 7 shows what

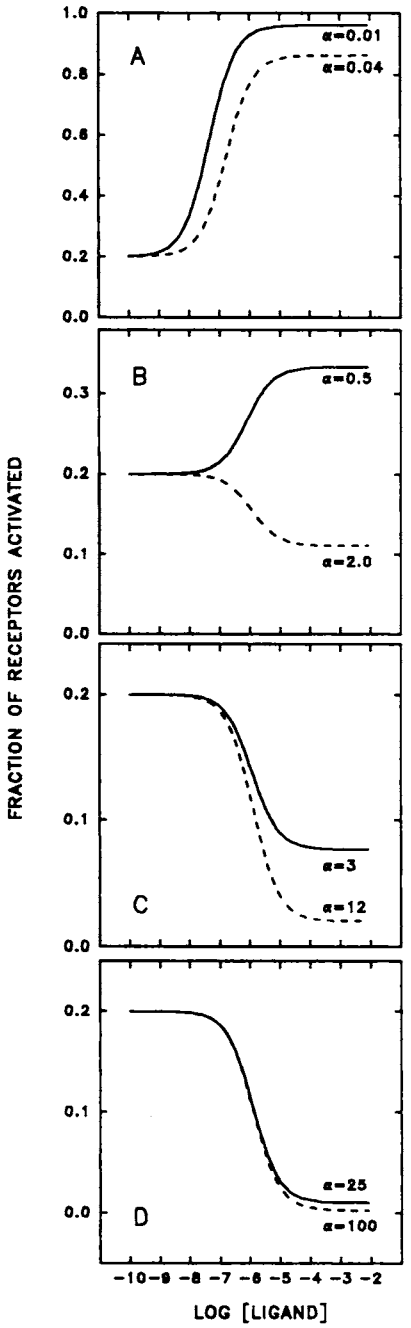

Fig. 7. Simulation of receptor regulation in terms of the two-state model. The receptor is presumed to isomerize between an inactive state (R) and an active state (R*), governed by the equilibrium constant K_I (K_I = [R]/[R*]). Ligand (L) binds to the inactive state with the dissociation constant K_L (K_L = [L][R]/[LR]) and to the active state with the dissociation constant αK_L (αK_L = [L][R*]/[LR*]). Isomerization of the liganded receptor is governed by the equilibrium constant αK_I (αK_I = [LR]/[LR*]). The fraction of receptors in the active state at a given [L] is defined as $(\alpha K_L + [L])/\{\alpha K_L(1 + K_I) + [L](1 + \alpha K_I)\}$. For all of the simulations shown, K_L = 1E − 6 and K_I = 4. *Solid curves*, ligand effects at various values of α; *dashed curves*, result of a 4-fold decrease in ligand selectivity for the active form of the receptor. A, A value of α = 0.01 allows for virtually complete receptor activation, consistent with a full agonist, whereas a value of 0.04 leads to a rightward shift and submaximal activation, a pattern typically observed after desensitization. B, A value of α = 0.5 yields a pattern consistent with weak partial agonism; increasing α to 2.0 leads to an inhibitory ligand effect. C, Increasing α from 3 to 12 increases the maximal inhibitory effect of a weak inverse agonist, whereas an equivalent increase (from 25 to 100) has little effect on a highly efficacious inhibitory ligand (D).

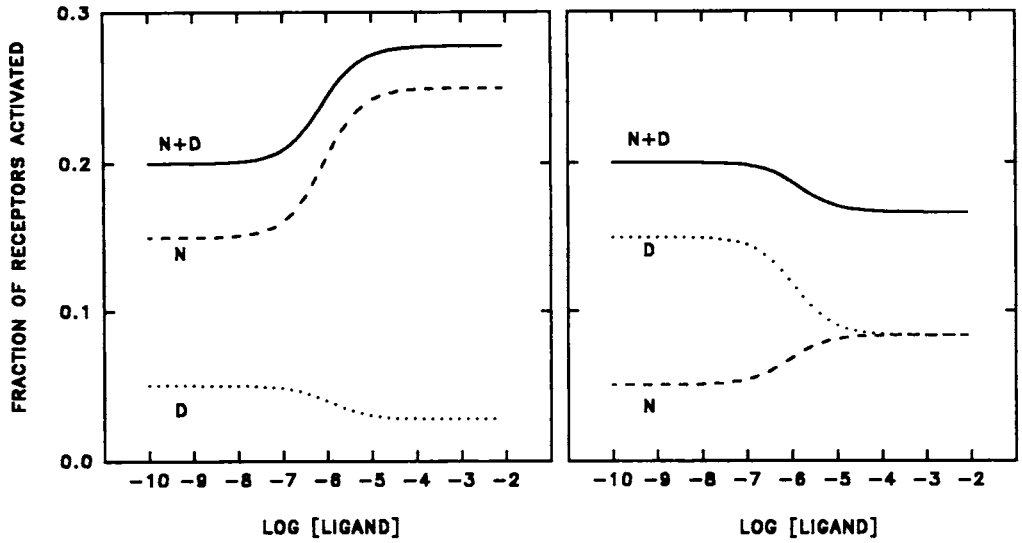

Fig. 8. Effects of altering the ratio of normal to desensitized receptors. The simulated data shown in Fig. 7B were taken to represent the effects of a poorly selective ligand on normal (α = 0.5) and desensitized (α = 2.0) receptors. *Left*, curves were generated by assuming a mixed population in which 25% of the receptors are in the desensitized state (*D*) and the remaining 75% are not (*N*), with the total response equal to the sum of the two curves (*N* + *D*). *Right*, curves were generated in an analogous manner, with the proportion of desensitized receptors increased to 75%.

would be expected in a two-state system after an increase (arbitrarily set at 4-fold) in the α value for an agonist (Fig. 7A), a weak partial agonist (Fig. 7B), a partial inverse agonist (Fig. 7C), and a full inverse agonist (Fig. 7D) with all other parameters being unchanged. Interestingly, this simulation qualitatively mimics the observed effects of desensitization: a weak partial agonist becomes a partial inverse agonist, a partial inverse agonist has its inverse efficacy increased, and a highly efficacious inverse agonist is relatively unaffected. It is noteworthy that this simulation is not associated with any change in the fraction of active receptor in the absence of ligand. This is consistent with our observation that no consistent changes in the basal ligand-independent adenylyl cyclase activity occur in the current system after desensitization.

This model also presents the advantage of providing a possible explanation for the variable effects of DCI (partial agonist versus inverse agonist) in control membranes. Indeed, a population of receptor in a biological system is presumably composed of a mixture of normal and desensitized receptors. Therefore, the observed signal with a given ligand would reflect the sum of its effects on the two receptor isoforms. If these two isoforms responded in manifestly different ways to a given ligand (dual ligand such as DCI; see Figs. 5 and 7B), then the net effect would be highly dependent on the relative amounts of normal and desensitized receptors in the initial conditions. Fig. 8 illustrates the simulation of the effects of a dual ligand on preparations expressing either 25% (Fig. 8A) or 75% (Fig. 8B) of desensitized receptors. These simulations clearly show that at some critical ratio of normal to desensitized receptors, the observed activity of the ligand would switch from being stimulatory (Fig. 8A) to being inhibitory (Fig. 8B). The two effects would cancel each other out only if they were exactly equal in magnitude, which might be a relatively rare event. This concept allows for a rationalization of the variable and bimodal effects of DCI in the control membranes (see Fig. 4A), which may contain differing proportions of desensitized receptors.

Although the two-state model allows simulations that are consistent with the data observed in the current study, other models could also be considered. In particular, desensitization might not change the relative affinities of the ligands for R and R* but rather may reduce the coupling efficacy of the ligand-occupied Rd* with its G protein without perturbation of the R-versus-(R* + Rd*) equilibrium. Many of the observations made in the current study could also be simulated by such a model (not shown). However, this model would predict that desensitization is accompanied by a reduction in the basal ligand-independent adenylyl cyclase activity. As previously indicated, no such reduction was observed, but it could reflect our inability to detect a very modest decrease. Obviously, additional studies are required to further test these two models and to provide a definitive mechanism of action that can account for the modulation of ligand efficacies after agonist-induced desensitization.

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