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DESENSITIZATION OF β-ADRENERGIC RECEPTOR-COUPLED ADENYLATE CYCLASE ACTIVITY

DIFFERENCES FOLLOWING EXPOSURE OF CELLS TO TWO FULL AGONISTS*

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Abstract—The effects on the β-adrenergic receptors of intact L6 muscle cells of exposure to agonists were investigated. Treatment of cells with isoproterenol decreased GTP-, isoproterenol-, and zinterol-stimulated adenylate cyclase activities, whereas exposure of cells to zinterol decreased only isoproterenol- and zinterol-stimulated activities. Although the effects of these two full agonists on GTP-stimulated adenylate cyclase activity were different, the time courses for development and reversal of the effects were similar. The decrease in agonist-stimulated adenylate cyclase activity observed in membranes prepared from cells previously exposed to isoproterenol or zinterol was due to both an increase in the concentration of agonist required for half-maximal activation and a decrease in the maximum level of activation. No effects on fluoride- or manganese-stimulated activities were observed following exposure of cells to either agonist. Decreases in GTP-stimulated adenylate cyclase activity were also observed following exposure of cells to epinephrine, Cc-25, fenoterol, norepinephrine, terbutaline, or metaproterenol, but not following incubation of cells with dobutamine or salmefamol. The results suggest that full agonists interacting with the same receptor can induce different changes in the components of the adenylate cyclase system. Desensitization following exposure to zinterol may result from modification of the receptor, whereas that following exposure to isoproterenol may be due to alterations in both the guanine nucleotide-binding protein and the receptor.

Exposure of cells to various hormones or drugs results in activation of adenylate cyclase. Following an initial increase in enzyme activity, continued exposure to an agonist results in desensitization characterized by a functional uncoupling of the receptor and a decrease in agonist-stimulated adenylate cyclase activity [1–7]. The decrease in adenylate cyclase activity occurring after brief periods of exposure to an agonist is not associated with a decrease in the density of receptors and occurs with a half-time of less than 5 min [1, 2, 4, 8].

The mechanisms responsible for the observed functional uncoupling of receptors and adenylate cyclase are being investigated currently in several laboratories. Exposure of 1321N1 astrocytoma cells to agonists causes alterations in the sedimentation properties of β-adrenergic receptors such that receptors appear to segregate in the membrane away from the guanine nucleotide-binding protein [9]. Studies with NRK-S cells [7] and the CYC⁻‖ variant of S49 cells [8, 10] suggest that a modification of the receptor occurs as a consequence of exposing cells to an agonist. Finally, in pigeon erythrocytes, the exchange reaction of guanine nucleotides is altered as a consequence of exposure to an agonist [11]. These mechanisms of uncoupling are not necessarily mutually exclusive and may occur simultaneously. In WI-38 fibroblasts, different forms of uncoupling appear to occur following exposure of cells in epinephrine and PGE_1 [4]. Therefore, different receptors can become uncoupled from adenylate cyclase by different mechanisms.

Previous work indicated that not all full agonists interact in the same way with β-adrenergic receptors on intact cells. For example, isoproterenol rapidly converts receptors to a form which has a low affinity for agonists, whereas zinterol does not convert receptors on intact cells to a low-affinity form [12]. This finding suggested that components of the receptor/adenylate cyclase system may be differentially modified following interaction with different agonists. In an attempt to gain a better understanding of possible mechanisms responsible for this low-affinity form of the receptor, and to determine if similar differences occurred during the uncoupling

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‖ Abbreviations: CYC⁻, cells with a functional deficiency in the guanine nucleotide-binding protein that serves to link receptor occupancy with activation of adenylate cyclase; PGE_1, prostaglandin E_1; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; [^{125}I] IHYP, [^{125}I]iodohydroxybenzylpindolol; and Gpp(NH)p, 5′-guanylyl imidodiphosphate.

of β-adrenergic receptors from adenylate cyclase, the present experiments were carried out.

EXPERIMENTAL PROCEDURES

Materials. GTP was purchased from Boehringer Mannheim (Indianapolis, IN). The following drugs were obtained as gifts: *l*-propranolol (Ayerst Laboratories, New York, NY); zinterol (Mead Johnson & Co., Evansville, IN); phentolamine and terbutaline (Ciba–Geigy Corp., Summit, NJ); salmefamol (Allen & Hanburys, Ware, U.K.); fenoterol and metaproterenol (Boehringer Ingelheim, Ltd., Elmsford, NY); hydroxybenzylpindolol (Sandoz Pharmaceuticals, Hanover, NJ); dobutamine (Eli Lilly & Co., Indianapolis, IN); and Cc-25 (N.V. Phillips Duphar, Weesp, Holland). All other drugs and reagents used were commercially available and were of reagent grade.

Cell culture. A nonfusing variant of the L6 muscle line originally developed by Yaffe [13] was used for these studies. Cells were grown in monolayer culture in Dulbecco's modification of Eagle's medium (Flow Laboratories) with 10% fetal calf serum (Sterile Systems, Inc.) and 50 μg/ml gentamycin in an atmosphere of 10% CO_2 and 90% air at 37°. Cells were plated at 15,000–20,000 cells/cm^2, fed on day 2, and either subcultured on day 3 using 0.1% trypsin in phosphate-buffered saline or harvested on days 5–6.

Incubation procedure and membrane preparation. Drugs were added to cultures of cells in the presence of normal growth medium. Incubations were performed at 37° in an incubator containing 10% CO_2. Cells in monolayer culture rinsed three times with ice-cold 1 mM sodium Hepes (pH 7.5) were osmotically disrupted in 10 ml of ice-cold 1 mM sodium Hepes by incubation at 4° for 15 min. Osmotically shocked cells were removed from culture dishes by scraping with a rubber policeman, and the lysate was centrifuged at 20,000 *g* for 10 min. Three centrifugations (20,000 *g*) and washes (1 mM sodium Hepes) were performed on membrane pellets. The second wash in some experiments contained 50 μM GTP to remove persistently bound agonist [14]. The final pellet was resuspended in 1 mM sodium Hepes and used in assays of β-adrenergic receptors or of adenylate cyclase activity.

Assay of adenylate cyclase activity. Adenylate cyclase activity was determined according to modifications [15] of the method of Salomon *et al.* [16]. Reactions were carried out in a final volume of 110–150 μl containing 50 mM sodium Hepes (pH 7.5), 0.5 mM EGTA, 1.5 mM $MgCl_2$, 1 mM 1-methyl-3-isobutylxanthine, 10 mM creatine phosphate, 0.1 mg/ml creatine kinase, 0.25 mM ATP, 1–3 × 10^6 cpm [α-^{32}P]ATP, and various drugs. GTP (30 μM), fluoride (10 mM), or manganese (10 mM) was included in some assays. Basal adenylate cyclase activity was measured in the presence of 30 μM GTP. Reactions were initiated by the addition of 50 μl of tissue (30 μg protein) and were carried out for 8 min at 37°.

GTP-stimulated adenylate cyclase activity was subtracted from agonist-stimulated activity when determining relative effects of agonists on adenylate cyclase activity and $T_{1/2}$ values for rates of change in enzyme activity. GTP-stimulated activity (basal) was not subtracted from the data shown in the figures.

Assay of β-adrenergic receptors. [^{125}I]Iodohydroxybenzylpindolol ([^{125}I]IHYP) was prepared [17, 18] and binding assays were performed as previously described [19].

Protein determination. Protein concentration was determined by the method of Bradford [20] using bovine serum albumin (Fraction V) as a standard.

RESULTS

Time course of changes in adenylate cyclase activity following short-term exposure to isoproterenol. Adenylate cyclase activity was measured in membranes prepared from cells exposed to 2.5 μM isoproterenol for various periods of time (Fig. 1). Manganese (Mn^{2+}) stimulation of adenylate cyclase activity was not affected by preincubation of cells with isoproterenol. Similarly, no significant change in fluoride-stimulated adenylate cyclase activity occurred as a result of exposure of cells to isoproterenol. A decrease in isoproterenol-stimulated activity was observed, with the maximum effect occurring after a 20-min exposure of intact cells to isoproterenol. A 69% decrease in GTP-stimulated adenylate cyclase activity was observed in studies with membranes prepared from cells exposed to isoproterenol. The half-time for the reduction in GTP-stimulated activity was less than 1 min, whereas the half-time for the decrease in isoproterenol-stimulated activity was about 5–6 min.

Stimulation of adenylate cyclase activity by zinterol in membranes prepared from cells exposed to isoproterenol decreased rapidly with a $T_{1/2}$ of about 2 min. In control tissues (zero time point), the intrin-

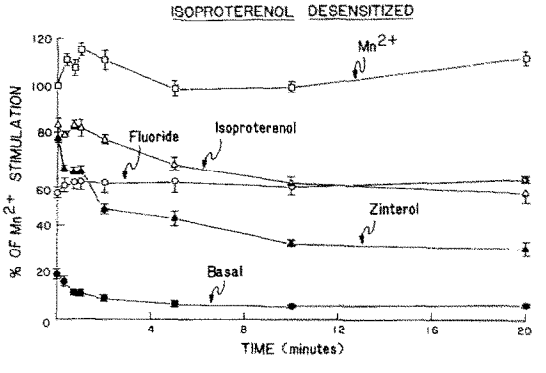

Fig. 1. Effect on adenylate cyclase activity of incubating cells with isoproterenol. Cells were exposed to isoproterenol (2.5 μM) for the indicated periods of time before membranes were prepared as described in Experimental Procedures. Adenylate cyclase activity was measured in the absence or presence of maximally effective concentrations of isoproterenol (50 μM), zinterol (5 μM), fluoride (10 mM), or manganese (10 mM). GTP (30 μM) was added to all reactions except those containing fluoride and manganese. Zero time refers to data for control cells not exposed to isoproterenol. Duplicate determinations were performed, and the data represent the mean ± S.E.M. of results obtained in five experiments. Results are expressed as a percentage of manganese-stimulated adenylate cyclase activity in control tissue.

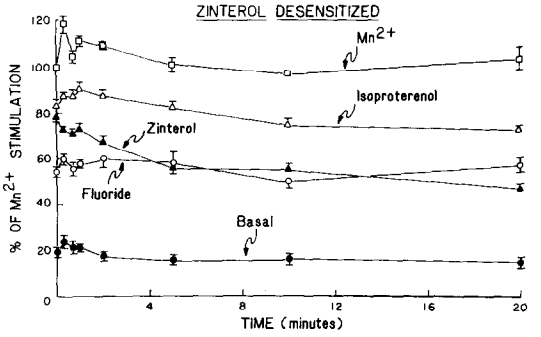

Fig. 2. Effect on adenylate cyclase activity of incubating cells with zinterol. Cells were exposed to zinterol (0.25 μM) for the indicated periods of time before preparation of membranes. Assays were performed and results are presented as described in Fig. 1. Zero time refers to data for control cells not exposed to zinterol. Duplicate determinations were performed, and the data represent the mean ± S.E.M. of results obtained in five experiments.

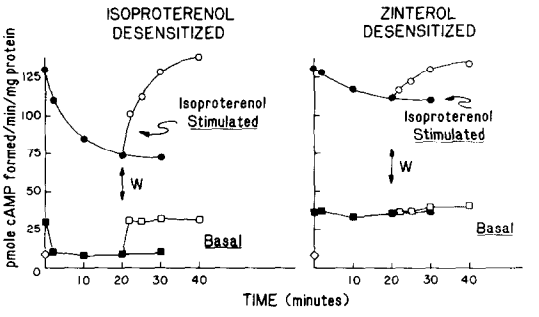

Fig. 3. Time course of the effect of exposure of cells to isoproterenol and zinterol on adenylate cyclase activity and recovery of activity following removal of drugs. Cells were exposed to isoproterenol (2.5 μM) or zinterol (0.25 μM) for the indicated periods of time before membranes were prepared. Adenylate cyclase activity was measured in the presence of 30 μM GTP (basal) or 30 μM GTP and 50 μM isoproterenol (isoproterenol-stimulated). In some experiments, drugs were removed after 20 min by washing three times with fresh medium (W). Cultures were then returned to the incubator. Cells were harvested at various times following this procedure (open symbols). Assays were performed as described in Experimental Procedures and in Fig. 1. Zero time refers to data for cells not exposed to an agonist. The open diamonds at zero time are the values for basal activity in membranes prepared from control cells in the absence of added GTP. Triplicate determinations were performed. The data are representative of three similar experiments.

sic activity of zinterol was 0.94 compared to isoproterenol. After incubating cells with isoproterenol for 20 min, the intrinsic activity of zinterol was reduced to 0.40 relative to that for isoproterenol. Both zinterol and isoproterenol appeared to stimulate adenylate cyclase activity only by interacting with β-adrenergic receptors since propranolol (0.3 μM) was able to inhibit the activity stimulated by either agonist (data not shown).

Time course of changes in adenylate cyclase activity following short-term exposure to zinterol. As observed with membranes prepared from cells exposed to isoproterenol, there was essentially no change in either Mn^{2+}-stimulated adenylate cyclase activity or fluoride-stimulated activity following exposure of cells to 0.25 μM zinterol for up to 20 min (Fig. 2). In contrast to the results observed following exposure of cells to isoproterenol, there was no change in basal activity following exposure to zinterol. In membranes prepared from cells exposed to zinterol, the half-time for the loss of isoproterenol-stimulated activity (a 10% loss) was about 10 min and the half-time for loss of zinterol-stimulated activity (a 40% loss) was approximately 3 min (Fig. 2).

Zinterol-stimulated adenylate cyclase activity decreased at a faster rate and to a greater extent than did isoproterenol-stimulated activity in membranes prepared from cells exposed to either isoproterenol or zinterol (Figs. 1 and 2). Maximal isoproterenol-stimulated adenylate cyclase activity (basal activity subtracted) was decreased more following exposure of cells to isoproterenol for 20 min than following exposure of cells to zinterol (77.5 ± 1.2% of control vs. 90.2 ± 0.7%). Pretreatment of L6 cells for 20 min with a combination of isoproterenol (2.5 μM) and zinterol (0.25 μM) elicited results similar to those observed with isoproterenol alone (data not shown).

Time course for reversal of desensitization. The decrease in adenylate cyclase activity observed following exposure of cells to either isoproterenol or zinterol was readily reversible following removal of drug and continued incubation of cells in drug-free medium (Fig. 3). Basal activity returned to control values rapidly following removal of isoproterenol and was complete within 2 min (Fig. 3, left). Isoproterenol-stimulated activity returned to control values with $T_{1/2}$ values of about 7 and 10 min for cells previously exposed to zinterol and isoproterenol respectively.

Dose-response relationship for isoproterenol-stimulated adenylate cyclase activity in control and desensitized membranes. The decrease in adenylate cyclase activity in membranes prepared from cells exposed to either isoproterenol or zinterol was characterized by a decrease in the maximal response (Fig. 4). Maximal isoproterenol-stimulated adenylate cyclase activity was decreased by 10 and 23% following exposure to zinterol and isoproterenol respectively (basal activity subtracted). A 5-fold decrease in the potency of isoproterenol for stimulation of adenylate cyclase activity was also observed in membranes prepared from cells exposed to isoproterenol (2.5 μM) or zinterol (0.25 μM) for 20 min (Fig. 4). The potency of zinterol was decreased by 2-fold following exposure of cells to either isoproterenol or zinterol (data not shown).

Gpp(NH)p activation of adenylate cyclase. The decrease in GTP-stimulated adenylate cyclase activity after desensitization with isoproterenol was investigated further by characterizing the temporal response to Gpp(NH)p (Table 1). Pretreatment of L6 cells with zinterol had no effect on the rate of activation or on the steady-state level of enzyme activity. Similarly, pretreatment with isoproterenol did not alter steady-state adenylate cyclase activity, but the apparent rate of activation was reduced by 50%.

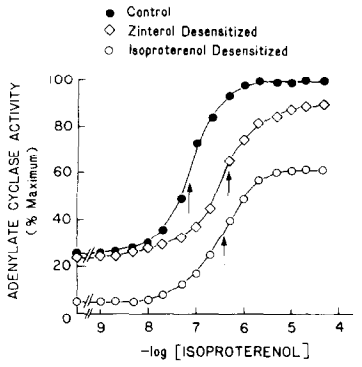

Fig. 4. Dose–response relationship for isoproterenol-stimulated adenylate cyclase activity. Cells were incubated with isoproterenol (2.5 μM) or zinterol (0.25 μM) for 20 min before membranes were prepared. Adenylate cyclase activity was determined in the presence of 30 μM GTP and various concentrations of isoproterenol. Duplicate determinations were performed. The data are representative of four similar experiments. Arrows indicate the concentration of drug which resulted in half-maximal activation of adenylate cyclase (K_{act}).

Table 2. Effect of desensitization by agonists on GTP-stimulated adenylate cyclase activity*

Drug	Conc (μM)	GTP-stimulated adenylate cyclase activity (% control)
Control		100
Salmefamol	2.5	105
Zinterol	0.25	99
Dobutamine	100.0	94
Cc-25	1.0	47
Terbutaline	100.0	39
Norepinephrine	100.0	38
Fenoterol	5.0	37
Metaproterenol	100.0	35
Isoproterenol	2.5	27
Epinephrine	5.0	25

* Cells were incubated with the concentration of drugs indicated (100 times their K_{act} values for cyclic AMP accumulation in intact cells) for 20 min before membranes were prepared. GTP-stimulated adenylate cyclase activity was determined in the presence of 30 μM GTP. Values represent the average of two separate experiments each performed in triplicate. The mean value for adenylate cyclase activity in control tissue was 20.3 pmoles of cyclic AMP formed/min/mg protein.

Effect of desensitization by agonists on basal adenylate cyclase activity. Exposure of cells to zinterol had little or no effect on GTP-stimulated adenylate cyclase activity in membranes, whereas exposure to isoproterenol resulted in a marked decrease in GTP-stimulated activity. To determine whether this effect was uniquely associated with either drug, cells were exposed for 20 min to eight other agonists (Table 2). Dobutamine had an intrinsic activity of 0.60, and all other drugs were full agonists (intrinsic activity greater than 0.90). Exposure of cells to zinterol, salmefamol, or dobutamine was not associated with decreases in GTP-stimulated activity, whereas exposure of cells to the other agonists resulted in a 50–75% decrease in GTP-stimulated activity measured in membrane preparations.

Effect of desensitization on binding of isoproterenol. The ability of isoproterenol to inhibit the binding of [^{125}I]IHYP was determined in membranes prepared from cells exposed to isoproterenol or zinterol for 20 min (Table 3). Exposure of cells to either agonist resulted in a marked decrease in the affinity of the receptor for isoproterenol. In agreement with previous studies [2, 3, 21], the affinity of the receptor for isoproterenol was similar in membranes prepared from treated cells and in membranes from control cells if assays were carried out in the presence of GTP.

DISCUSSION

The process of desensitization of the β-adrenergic or PGE_1 receptor/adenylate cyclase system appears to involve multiple steps [1–5, 23–25]. The initial step is thought to result from a reversible modification

Table 1. Activation of adenylate cyclase by Gpp(NH)p after desensitization with isoproterenol or zinterol*

	Adenylate cyclase activity (pmoles/mg/min) Initial	Steady-state	Apparent k (sec^{-1})
Control	171 ± 27.4	469 ± 10.0	0.76 ± 0.087
Isoproterenol-desensitized	139 ± 1.3	478 ± 1.6	0.37 ± 0.105†
Zinterol-desensitized	213 ± 21.5	411 ± 53.5	0.86 ± 0.336

* The time course for activation of adenylate cyclase by 30 μM Gpp(NH)p was determined in membranes prepared from cells incubated for 20 min with either 2.5 μM isoproterenol or 0.25 μM zinterol. A quasi-Newton method with difference approximations to derivatives was used with the aid of a CDC 6000 series computer to fit the data to the equation $V_t = V_s - (V_s - V_i)e^{-kt}$, where V_t is enzyme activity at time t, V_s is steady-state enzyme activity, V_i is initial activity, and k is an apparent rate constant characterizing the transition from V_i to V_s. Data are expressed as the mean ± S.E.M. of three to five separate experiments.

† $P < 0.05$ (Student's t-test) compared to control.

Table 3. Effect of desensitization by agonists on the affinity of β-adrenergic receptors for isoproterenol*

Treatment	K_D of receptor for isoproterenol (μM) −GTP	+GTP
Control	0.026 ± 0.004	0.58 ± 0.06
Isoproterenol-treated	0.21 ± 0.03	0.70 ± 0.02
Zinterol-treated	0.14 ± 0.03	0.85 ± 0.10

* The EC_{50} of isoproterenol was determined in membranes prepared from control cells or cells exposed to isoproterenol (2.5 μM) or zinterol (0.25 μM) for 20 min. Fifteen concentrations of isoproterenol were used to inhibit the binding of a constant amount of [^{125}I]IHYP (50–60 pM). Experiments were carried out in the absence or presence of 300 μM GTP. EC_{50} Values were converted to K_D values by the method of Cheng and Prusoff [22]. Data represent the mean ± S.E.M. of three experiments.

of the receptor and/or guanine nucleotide-binding protein such that there is a decreased "coupling" between components of the system. The uncoupling process results in a rapid decrease in agonist-stimulated adenylate cyclase activity. It has been shown for both β-adrenergic receptors [1–3] and PGE_1 receptors [4] that uncoupling is reversible if agonists are removed; however, if the integrity of the cells is disrupted, the receptors appear to be irreversibly uncoupled from other components of the adenylate cyclase system.

In the present study, exposure of cells to either zinterol or isoproterenol resulted in a decrease in the affinity of the β-adrenergic receptor for isoproterenol as measured by inhibition of the binding of [^{125}I] IHYP. Part of the effect of agonists on the adenylate cyclase system, therefore, may be due to a modification of the β-adrenergic receptor. Similar effects of agonists on the properties of β-adrenergic receptors have been reported previously [2, 3, 21]. The density of β-adrenergic receptors did not decrease, however, following exposure of cells for up to 60 min to either isoproterenol or zinterol (unpublished observations). Therefore, the observed decrease in adenylate cyclase activity was not due to a decrease in the density of receptors.

The decrease in agonist-stimulated adenylate cyclase activity could be the result of an increase in the concentration of agonist required for half-maximal stimulation or of a decrease in maximum activity. In many systems, only an effect on maximum activity has been observed [2, 4, 6, 11]; however, increased K_{act} values have been reported following desensitization with PGE_1 [5, 26] and following exposure of C6 glioma cells or S49 lymphoma cells to agonists at β-adrenergic receptors [5, 8]. In the present study, incubation of cells with isoproterenol resulted in a decrease in maximum activity and an increase in the K_{act} value.

Exposure of cells to agonists did not alter the fluoride-stimulated or the steady-state level of Gpp(NH)p-stimulated adenylate cyclase activity. Since both agents activate adenylate cyclase through an effect on the guanine nucleotide-binding protein [27, 28], exposure of calls to agonists did not appear to affect the extent of activation of the guanine nucleotide-binding protein. Rather, both GTP-stimulated adenylate cyclase activity and the apparent rate of enzyme activation by Gpp(NH)p were decreased by pretreatment with isoproterenol, but not with zinterol, suggesting an isoproterenol-induced decrease in the rate at which the guanine nucleotide-binding protein is activated by guanine nucleotides. Likewise, Mn^{2+}, thought to activate adenylate cyclase by direct action on the catalytic subunit [29–31], had effects on adenylate cyclase activity in membranes prepared from cells exposed to agonists that were similar to those observed in control membranes. Therefore, a decrease in catalytic capacity was not responsible for the decrease in agonist-stimulated enzyme activity.

Exposure of WI-38 fibroblasts to PGE_1 is characterized by reversible decreases in GTP-, Gpp(NH)p-, epinephrine-, and PGE_1-stimulated adenylate cyclase activity [4]. Incubation of WI-38 fibroblasts with epinephrine, on the other hand, affected only epinephrine-stimulated adenylate cyclase activity [4]. The authors interpreted this to suggest that desensitization following exposure to epinephrine resulted in modification of the receptor, but desensitization following incubation with PGE_1 resulted in modification of the guanine nucleotide-binding protein. Whereas in WI-38 cells different effects were observed following exposure of cells to agonists interacting with different receptors, in the present study different effects were observed with two full agonists interacting with the same receptor. A large decrease in GTP-stimulated activity was observed in cells exposed to isoproterenol, but not in cells exposed to zinterol. This differential effect was also observed with a number of other agonists. Thus, desensitization following exposure to isoproterenol may result from modification of the guanine nucleotide-binding protein (and possibly the receptor), whereas desensitization following exposure to zinterol, salmefamol, or dobutamine resulted only in modification of the receptor.

Since NaF- and Gpp(NH)p-stimulated adenylate cyclase activities were not altered, desensitization with isoproterenol does not reduce the extent of activation of the guanine nucleotide-binding protein. Rather, pretreatment with isoproterenol, but not with zinterol, appears to decrease the rate of activation of the guanine nucleotide-binding protein.

It is possible that not all of the zinterol was washed out of the membranes and this could result in an artifactually high level of GTP-stimulated activity. This seems unlikely because an additional two washes of membrane pellets did not affect GTP-stimulated activities in membranes from cells exposed to isoproterenol or zinterol. In addition, the inclusion of GTP in the buffer used for the preparation of membranes did not affect GTP- or Gpp(NH)p-stimulated activity in membranes prepared from desensitized cells. This latter manipulation should remove any zinterol bound to the receptor [14, 32, 33].

The differences in the effects observed following exposure of cells to isoproterenol and zinterol demonstrate that two drugs interacting with the same receptor can exhibit different mechanisms of desensitization. Since these drugs induce similar increases

in cyclic AMP, it is unlikely that the effects of isoproterenol on GTP-stimulated adenylate cyclase activity require cyclic AMP-dependent protein kinase.

REFERENCES

1. Y-F. Su, T. K. Harden and J. P. Perkins, *J. biol. Chem.* **254**, 38 (1979).
2. Y-F. Su, T. K. Harden and J. P. Perkins, *J. biol. Chem.* **255**, 7410 (1980).
3. T. K. Harden, Y-F. Su and J. P. Perkins, *J. Cyclic Nucleotide Res.* **5**, 99 (1979).
4. R. B. Clark and R. W. Butcher, *J. biol. Chem.* **254**, 9373 (1979).
5. V. Homburger, M. Lucas, B. Cantau, J. Barabe, J. Penit and J. Bockaert, *J. biol. Chem.* **255**, 10436 (1980).
6. I. Morishima, W. J. Thompson, G. A. Robison and S. J. Strada, *Molec. Pharmac.* **18**, 370 (1980).
7. A. Levitzki and D. Atlas, *Life Sci.* **28**, 661 (1981).
8. D. A. Green, J. Friedman and R. B. Clark, *J. Cyclic Nucleotide Res.* **7**, 161 (1981).
9. T. K. Harden, C. U. Cotton, G. L. Waldo, J. K. Lutton and J. P. Perkins, *Science* **210**, 441 (1980).
10. D. A. Green and R. B. Clark, *J. biol. Chem.* **256**, 2105 (1981).
11. T. H. Hudson and G. L. Johnson, *Molec. Pharmac.* **20**, 694 (1981).
12. R. N. Pittman and P. B. Molinoff, *Molec. Pharmac.* **24**, 398 (1983).
13. D. Yaffe, *Proc. natn. Acad. Sci. U.S.A.* **61**, 477 (1968).
14. K. A. Heidenreich, G. A. Weiland and P. B. Molinoff, *J. Cyclic Nucleotide Res.* **6**, 217 (1980).
15. K. P. Minneman, L. R. Hegstrand and P. B. Molinoff, *Molec. Pharmac.* **16**, 21 (1979).
16. Y. Salomon, C. Londos and M. Rodbell, *Analyt. Biochem.* **58**, 541 (1974).
17. M. E. Maguire, R. A. Wiklund, H. J. Anderson and A. G. Gilman, *J. biol. Chem.* **251**, 1221 (1976).
18. T. K. Harden, B. B. Wolfe and P. B. Molinoff, *Molec. Pharmac.* **12**, 1 (1976).
19. R. N. Pittman and P. B. Molinoff, *J. Cyclic Nucleotide Res.* **6**, 421 (1980).
20. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
21. J. M. Stadel, A. DeLean, D. Mullikin-Kilpatrick, D. D. Sawyer and R. J. Lefkowitz, *J. Cyclic Nucleotide Res.* **7**, 37 (1981).
22. Y-C. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
23. Y-F. Su, X. L. Cobeddu and J. P. Perkins, *J. Cyclic Nucleotide Res*, **2**, 257 (1976).
24. Y-F. Su, G. L. Johnson, X. L. Cubeddu, B. H. Leichtling, R. Ortmann and J. P. Perkins, *J. Cyclic Nucleotides Res.* **2**, 271 (1976).
25. R. Barber, R. B. Clark, L. A. Kelly and R. W. Butcher, *Adv. Cyclic Nucleotide Res.* **9**, 507 (1978).
26. S. R. Ayad and G. R. J. Burns, Expl. Cell Res. **118**, 229 (1979).
27. N. Kimura and N. Nagata, *J. biol. Chem.* **252**, 3829 (1977).
28. T. Pfeuffer, *J. biol. Chem.* **252**, 7224 (1977).
29. E. M. Ross, A. C. Howlett, K. M. Ferguson and A. G. Gilman, *J. biol. Chem.* **253**, 6401 (1978).
30. A. C. Howlett, P. C. Sternweis, B. A. Macik, P. M. Van Arsdale and A. G. Gilman, *J. biol. Chem.* **254**, 2287 (1979).
31. W. Schlegel, E. S. Kempner and M. Rodbell, *J. biol. Chem.* **254**, 5168 (1979).
32. J. M. Stadel, A. DeLean and R. J. Lefkowitz, *J. biol. Chem.* **255**, 1436 (1980).
33. L. E. Limbird, D. M. Gill and R. J. Lefkowitz, *Proc. natn. Acad. Sci. U.S.A.* **77**, 775 (1980).