Catecholamine-Induced Alteration in Sedimentation Behavior of Membrane Bound β-Adrenergic Receptors

Abstract. *Incubation of astrocytoma cells with catecholamines results in a decrease in catecholamine-stimulated adenylate cyclase activity and a concomitant alteration in the sedimentation properties of particulate β-adrenergic receptors. The altered receptors exhibit agonist binding properties similar to those of receptors that are "uncoupled" from adenylate cyclase.*

Incubation of target cells with hormones results in many systems in a reduction in responsiveness of cells to subsequent exposure to appropriate agonists. Catecholamine-induced desensitization of adenylate cyclase in human astrocytoma cells (1321N1) involves at least two reactions. First, upon exposure of cells to isoproterenol a rapid ($t_{1/2} \approx 3$ minutes) functional uncoupling of β-adrenergic receptors from adenylate cyclase occurs. This reaction is expressed as a 50 to 60 percent decline in the responsiveness of the enzyme to isoproterenol and a decrease in the apparent affinity of the receptor for isoproterenol (*1, 2*). A second reaction, which occurs after a lag of about 60 minutes, results in a loss of β-adrenergic receptors (*2, 3*). The first reaction is rapidly reversible ($t_{1/2} \approx 7$ minutes) upon removal of isoproterenol (*1, 2*), whereas the generation of lost receptors occurs slowly, if at all (*2*).

Incubation of target cells with peptide hormones results in the internalization of bound hormone (*4, 5*). Although it is difficult to demonstrate unequivocally internalization of the plasmalemma receptor, such a mechanism has been proposed to mediate agonist-induced down regulation of peptide hormone receptors (*5*). Direct evidence for a similar mechanism in the regulation of responsiveness of cells to catecholamines is not available. However, Chuang and Costa (*6*) reported that catecholamines cause the appearance of small amounts of "soluble" β-adrenergic receptors in the cytosol fraction of frog erythrocytes.

We report that incubation of 1321N1 astrocytoma cells with isoproterenol results in the accumulation of a subpopulation of β-adrenergic receptors that exhibit altered sedimentation properties. These receptors exhibit the agonist-binding characteristics of "uncoupled" receptors.

Our initial studies involved an analysis of the distribution of β-adrenergic receptors of 1321N1 cell lysates on sucrose density gradients. As is shown in Fig. 1A, β-adrenergic receptors, measured by the binding of ^{125}I-labeled hydroxybenzylpindolol (HYP) (*7*), sediment as a wide band on sucrose density gradients of lysates of control cells. The distribution of sodium fluoride- and isoproterenol-stimulated adenylate cyclase activities was similar to that of β-adrenergic receptors. We have shown (*1-3*) that short-term (5 to 30 minutes) incubation of 1321N1 cells with 1 μ*M* isoproterenol results in a 50 to 60 percent loss of isoproterenol-stimulated adenylate cyclase activity, with no change occurring in basal adenylate cyclase activity, in sodium fluoride-, guanyl-5′-yl imidodiphosphate-, or prostaglandin E_1-stimulated adenylate cyclase activities, or in the number of β-adrenergic receptors. A 15-minute incubation of 1321N1 cells with 1 μ*M* isoproterenol also markedly alters the distribution of β-receptors on sucrose density gradients (Fig. 1A), with ^{125}I-labeled HYP binding activity now predominately migrating to less dense fractions of the gradient. Under this condition there was no qualitative change in the pattern of distribution of isoproterenol-stimulated adenylate cyclase activity, although the activity was reduced 50 to 60 percent in desensitized cells.

Recently, the method of Scarborough (*8*), in which concanavalin A (con A) is used, has been adapted for the preparation of plasma membranes from 1321N1 astrocytoma cells (*9*). The apparent basis of the usefulness of con A in this method involves a cross-linking reaction that stabilizes the plasma membrane to fragmentation and vesiculation during cell lysis (*8*). Treatment of cells with con A results in the formation of plasma membrane fragments that migrate as more uniform particles to heavier densities on sucrose density gradients upon centrifugation (*8, 9*). In the presence of con A, β-adrenergic receptors and adenylate cyclase activity migrate predominately as a single sharp band at a density of 45 to 50 percent sucrose (*9*). Since con A treatment of cells results in a more effective density gradient separation of plasma membranes from other cellular components, the distribution of β-adrenergic receptors and adenylate cyclase was assessed on sucrose density gradients after treatment with con A of control and desensitized cells. The distribution of β-adrenergic receptors on these gradients was also markedly altered by incubation of cells for 15 minutes with 1 μ*M* isoproterenol (Fig. 1B). Although the total number of receptors was unchanged on these gradients, there was a significant increase in the number of receptors in lighter fractions (30 to 35 percent sucrose) of the gradient and a corresponding decrease in the peak at 45 to 50 percent sucrose. Again, this change occurred with a 50 to 60 percent decrease in isoproterenol-stimulated adenylate cyclase activity in cell lysates, but with no apparent alteration in the gradient distribution of the remaining activity (Fig. 1C). The alteration in the gradient distribution of β-adrenergic receptors was apparent as early as 5 minutes after incubation in 1 μ*M* isoproterenol. The alteration was rapidly reversible since incubation of desensitized cells for 30 minutes in the absence of catecholamines resulted in the complete recovery of isoproterenol-stimulated adenylate cyclase activity to control levels (*1, 2*) and in a gradient distribution of receptors that was indistinguishable from control.

The designation of the early component of catecholamine-induced desensitization as an uncoupling was based in part on a change in the agonist-binding properties of β-adrenergic receptors in desensitized cells (*1, 2*). Receptors of control cells exhibit a high affinity for isoproterenol in the absence of guanosine triphosphate (GTP) and a 10- to 50-fold lower affinity in the presence of GTP. The β-adrenergic receptors of cells exposed to isoproterenol for 15 to 30 minutes exhibit a markedly lower affinity for isoproterenol in the absence of GTP and, as a result, GTP effects a smaller reduction in the apparent affinity for catecholamine than that observed for receptors of control cells (*1, 2*). Such binding behavior also has been observed in S49 lymphoma cell variants that are genetically uncoupled due to a deficiency or alteration of a nucleotide binding protein (or proteins) that appears to be necessary for the formation of a high-affinity receptor complex (*10*). In light of these studies, we examined the agonist-binding properties of the β-adrenergic receptors in both the light and heavy fractions of gradients prepared from lysates of control cells (Fig. 2A) and cells exposed to isoproterenol for 15 minutes (Fig. 2B). The β-adrenergic receptors from the light gradient fractions from both control and desensitized cells appeared to be uncoupled. That is, only low-affinity binding of isoproterenol was observed, and GTP had no apparent effect. In contrast, the agonist-binding properties of receptors in heavy gradient fractions were essentially identical to those observed in control lysates. That is, in the absence of

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GTP, the inhibition constant (K_i) for isoproterenol was 15 to 60 nM; in the presence of GTP the apparent affinity was reduced to 200 to 600 nM.

From our kinetic studies (*2*) we concluded that the uncoupling component of agonist-induced desensitization involved a change in the properties of β-adrenergic receptors without alterations in other components of the adenylate cyclase system. Within the limitations of our analysis, the agonist-induced changes in the sedimentation properties of this multicomponent enzyme system also appeared to be selective for β-adrenergic receptors. That is, no change occurred in either the distribution or amount of basal or sodium fluoride–stimulated adenylate cyclase activity, and the change in sedimentation of the β-adrenergic receptors appeared to be selective for a subpopulation that exhibits the properties of uncoupled receptors. Generally, exposure (15 minutes at 37°C) of the cells to 1 μM isoproterenol reslted in a 50 to 60 percent reduction in responsiveness of adenylate cyclase to catecholamines, a 40 to 60 percent reduction in the number of β-adrenergic receptors migrating on the gradient with adenylate cyclase, and a corresponding increase in receptors sedimenting at low densities of sucrose. In addition, the time courses of occurrence of the uncoupling process and the physical change in receptors appear to be similar. Although they have not been directly correlated in the same experiment, both events are clearly evident after 5 minutes of exposure of cells to isoproterenol, both processes reach an apparent steady state between 15 and 30 minutes, and both effects disappear after incubation of desensitized cells for 30 minutes in the absence of isoproterenol.

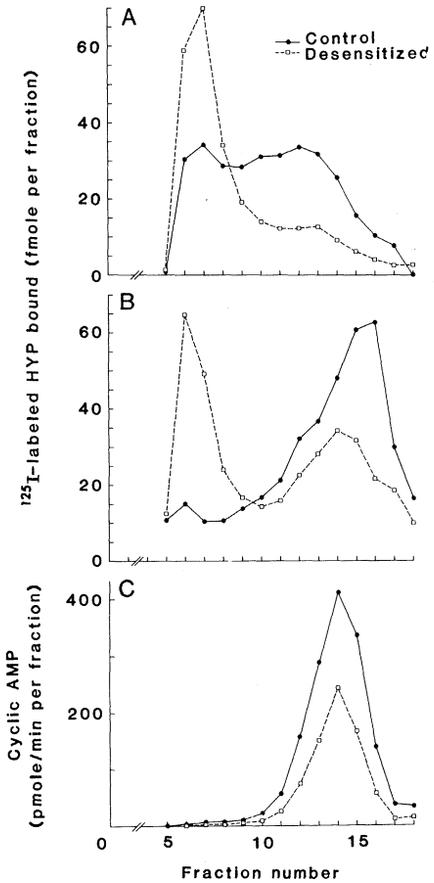

Fig. 1. Sucrose density gradient distribution of β-adrenergic receptors and adenylate cyclase after short-term incubation of cells with isoproterenol. (A) Cells were incubated with 1 mM sodium ascorbate (control) or 1 mM sodium ascorbate plus 1 μM isoproterenol (desensitized) for 15 minutes in normal growth medium. Cells were then lysed and centrifuged in a sucrose density gradient (*15*). The β-adrenergic receptor density was determined as described (*16*). The data represent three similar experiments. (B and C) Cells were treated as described above except that after incubation with isoproterenol, the cells were treated with con A before lysis (*15*). The ^{125}I-labeled HYP was used to determine β-receptor density (B) in gradient fractions. Isoproterenol-stimulated adenylate cyclase activity (C) was determined (*16*) in gradient fractions. The data represent six similar experiments; *AMP*, adenosine monophosphate.

Whether the change in the gradient profile of receptors is causal in the uncoupling process or occurs as a result of this event is not known; nor is the physiological significance of multiple forms of the β-adrenergic receptor understood. The sucrose gradient behavior of the uncoupled receptors suggests that, due either to physical inaccessibility or lack of con A receptor sites, the sedimentation properties of the membrane containing such receptors are not influenced by con A. It is possible that exposure of cells to catecholamines causes a portion of the β-adrenergic receptors to aggregate on the cell surface in regions that cannot be stabilized with con A. Alternatively, the uncoupled receptors could exist in cytoplasmic vesicles resulting from an agonist-induced selective endocytosis similar to the process proposed for internalization of other hormone-receptor complexes (*5*). We have not rigorously established that the uncoupled receptors exist in vesicles; however, their retention by glass fiber filters and their sedimentation behavior indicate that they exist in a particulate form. Experiments described elsewhere (*11*) establish that enzyme markers for the Golgi apparatus, lysosomes, and endoplasmic reticulum are concentrated in the light density fractions containing uncoupled receptors.

Although catecholamine-induced desensitization occurs in a variety of systems (*12, 13*), the general applicability of the phenomenon observed in our study is not yet known. In a number of systems (*12*), catecholamine-induced desensitization of adenylate cyclase is hormone-specific, and the occurrence of changes in the adenylate cyclase system are similar to the changes that occur in 1321N1 astrocytoma cells. Refractoriness of adenylate cyclase in rat C6-2B glioma cells, however, exhibits very different properties and appears to occur by a distinctly different process; that is, protein synthesis is required and changes in β-adrenergic receptors are not involved (*13*).

Evidence from several hormone-receptor systems suggests that agonists induce an internalization process that may eventually result in the degradation of cell surface receptors (*4, 5*). If such a process is induced in 1321N1 cells by isoproterenol, it could potentially explain our observations and account for the lag in time between the uncoupling phase of desensitization and the loss of β-adrenergic receptors (*1–3*). In this regard, the catecholamine-induced appear-

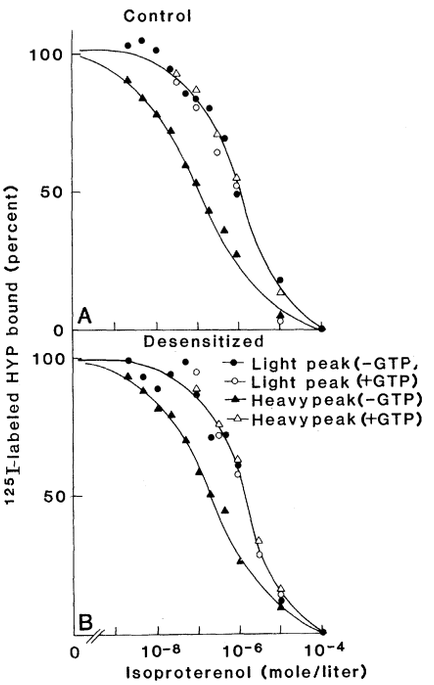

Fig. 2. Isoproterenol binding curves for β-adrenergic receptors isolated from light and heavy fractions of sucrose density gradients. Sucrose density gradients similar to that in Fig. 1B were generated from lysates of control and desensitized cells. Light fractions (fractions 6 and 7) and heavy fractions (fractions 14, 15, and 16) were pooled and processed as is described (*17*). Binding assays were performed with the use of ^{125}I-labeled HYP (*16*). Data are expressed as percent of control specific binding, which was 70 to 98 percent of total binding. The GTP concentration was 100 μM. The data are the means of three experiments in which ^{125}I-labeled HYP binding at each concentration of isoproterenol was determined in triplicate or quadruplicate.

ance of soluble β-adrenergic receptors in the cytoplasm of frog erythrocytes (*6*) may be directly related to the phenomenon observed in astrocytoma cells.

A small number of β-adrenergic receptors (usually < 10 percent of the total) appear in the light gradient fractions of cells that have not been exposed to isoproterenol (Fig. 1B). Under control conditions the agonist-binding properties of the β-adrenergic receptors in light gradient fractions are characteristic of uncoupled receptors; that is, they exhibit only low-affinity binding for isoproterenol in the absence or presence of GTP (Fig. 2). The existence of β-adrenergic receptors in the light fraction from control cells could represent a normal transient population of receptors that were recently synthesized, that are about to be degraded, or that are simply undergoing a process of membrane recyclization (*14*). It is not unreasonable to speculate that the natural turnover of the β-adrenergic receptor would involve a population of vesicle-bound receptors in the cytosol; desensitization, by changing the properties of the β-adrenergic receptor, could accelerate the degradative phase of such a process.

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15. The 1321N1 cells were grown on 150-mm glass or plastic culture dishes in Dulbecco's modification of Eagle's medium supplemented with 5 percent fetal calf serum. Incubation was in an atmosphere of 95 percent air and 5 percent CO_2 at 37°C in a humidified incubator. In most experiments, four to seven confluent 150-mm dishes were used per gradient. After incubation, the cells were washed with ice-cold serum-free growth medium containing 20 m*M* Hepes, *p*H 7.5. In some experiments (Fig. 1, B and C), 10 ml of growth medium containing 20 m*M* Hepes (*p*H 7.5) and con A (0.25 mg/ml) was added to each dish. After a 30-minute incubation at room temperature (18° to 19°C), con A–containing medium was removed and replaced with 10 ml of ice-cold 1 m*M* tris buffer, *p*H 7.5. After a 10- to 15-minute incubation on ice, the swollen cells were lysed in a small volume (1 to 2 ml per dish) by gently scraping with a rubber spatula. Continuous sucrose density gradients (28 ml) running from 30 to 55 percent (weight to volume) were formed above a 2-ml cushion of 60 percent sucrose; an ISCO model 570 gradient former was used. Cell lysate (9 ml) was layered on each gradient, and the gradients were centrifuged for 1 hour at 25,000 rev/min in a Beckman SW27 rotor and Beckman model L5-65 ultracentrifuge. Two-milliliter gradient fractions were collected in an ISCO model 568 fractionator.
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17. For experiments in which agonist binding curves were generated, fractions from several gradients were combined as follows. Light gradient fractions (fractions 6 and 7) and heavy gradient fractions (fractions 14, 15, and 16) were pooled from two control gradients and two gradients from desensitized cells. The pooled fractions were diluted five- to tenfold with 1 m*M* tris, *p*H 7.5, at 0°C and centrifuged in a SW27 rotor at 110,000*g* for 30 minutes. The supernatant was aspirated, the pellet was resuspended in 1 m*M* tris, *p*H 7.5, and the centrifugation was repeated. The pellet from this centrifugation was resuspended in 0.145*M* NaCl, 20 m*M* tris, *p*H 7.5, at 0°C, and centrifuged a third time before final resuspension in the same buffer.
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Flight Activity Initiated via Giant Interneurons of the Cockroach: Evidence for Bifunctional Trigger Interneurons

Abstract. *Activity in dorsal giant interneurons of the cockroach initiates flight movements if leg contact with a substrate is prevented. The same interneurons initiate activity associated with running when leg contact is maintained. Thus, which one of two completely different behaviors the giant interneurons evoke depends on the presence or absence of leg contact.*

Many behaviors are gated or triggered by activity in single interneurons or in discrete populations of interneurons. Such command or trigger interneurons have been identified in a wide variety of animals including annelids, arthropods, mollusks, and turtles (*1*).

An implication of these findings is that the excitation of a given command interneuron (or set of interneurons) automatically determines which behavior will be generated. In other words, each interneuron controls one and only one pattern of motor outputs. Although this might be the simplest way to control behavior, in many cases a much more efficient system would use multifunctional command-trigger interneurons; that is, the particular behavior initiated by an interneuron would depend on conditions that exist at that time.

One type of multifunctional command interneuron can evoke different behaviors by varying the frequency of its action potentials (*2*). Alternatively, a command interneuron producing a single pattern of activity could evoke multiple behaviors if existing physiological conditions, other than the command input, determine the appropriate behavior. Under the second system, a particular sensory stimulus consistently evokes different behaviors under different circumstances. For example, in the cockroach *Periplaneta americana*, wind puffs directed at the cerci (two antenna-like projections on the abdomen) initiate either running or flying, the type of movement depending on whether or not leg contact is made with a substrate (*3*). If the wind puff is presented while leg contact is maintained, the cockroach turns away from the wind source and runs (*4*, *5*). However, when leg contact is absent, a wind puff initiates flight (*3*) (Fig. 1B); that is, the wings unfold and beat at about 20 Hz. The legs are held up against the animal's abdomen rather than making running movements.

The giant interneurons (GI's) are prime candidates for triggering both of these behaviors. They are excited by sensory neurons that innervate wind receptive hairs on the cerci (*6*). Moreover, an excitatory pathway exists between many of the GI's and the leg neurons involved in walking movements (*7*). The motor activity initiated when each GI is excited fits well with movements observed in behavioral experiments with free-ranging animals (*5*).

All of these data provide strong circumstantial evidence supporting the role of the GI's in initiating running movements. If the same GI's that initiate these patterns also generate flight movements when leg contact is removed, the GI's will be established as bifunctional trigger

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