0026-895X/86/060521-10$02.00/0

MOLECULAR PHARMACOLOGY, 29:521-530



# Agonist-Mediated Regulation of $\alpha_1$- and $\beta_2$-Adrenergic Receptor Metabolism in a Muscle Cell Line, BC3H-1

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Received October 28, 1985; Accepted March 7, 1986

**SUMMARY**

We have compared the metabolism of $\alpha_1$- and $\beta_2$-adrenergic receptors whch are both expressed in BC3H-1 muscle cells. During growth of the cells to confluence, the number of $\alpha_1$-receptors per mg of membrane protein increases, whereas that of the $\beta_2$-receptors remains constant. Experiments using cycloheximide and irreversible $\alpha_1$- and $\beta_2$-receptor antagonists, phenoxybenzamine and *N*-[2-hydroxy-3-(1-naphthoxy)-propyl]-*N'*-bromoacetylethylenediamine, respectively, yield disparate turnover rates ($t_{1/2}$) for the two receptors: $\alpha_1$ ≃25 hr, $\beta_2$ ≃200 hr. These experiments suggest that synthesis of $\beta_2$-receptors virtually ceases in confluent cells. Maximally effective doses of agonists down-regulated both receptor types 80–90% and enhanced the rates of loss of both receptors ($t_{1/2}$ = 1–5 hr). The rates of down-regulation were not affected by cycloheximide, implying that agonists enhance receptor clearance rather than decrease receptor appearance. The rank orders of potencies of agonists for promoting receptor down-regulation were those characteristic of $\alpha_1$- and $\beta_2$-receptors. However, concentrations of agonists that resulted in down-regulation of each receptor subtype were 10- to 100-fold lower than those required for occupancy of receptors as assessed in radioligand binding studies. Receptor recovery following removal of agonists was blocked by cycloheximide and was much faster than the recovery that followed treatment of cells with irreversible antagonists. Therefore, protein synthesis (but perhaps not receptor synthesis per se) appears necessary for recovery from down-regulation. In addition, the rates of recovery of $\alpha_1$- and $\beta_2$-receptor-mediated functions (phosphatidylinositol turnover and cyclic AMP synthesis, respectively) following receptor down-regulation or irreversible blockade parallel the rates of receptor recovery. These data indicate that basal metabolism of $\alpha_1$- and $\beta_2$-receptors in BC3H-1 cells is substantially different, but that agonist-mediated changes in metabolism of the two receptor subtypes are similar. Thus, common mechanisms appear to mediate the regulation by agonists of $\alpha_1$- and $\beta_2$-receptors in these cells.

Agonist-specific desensitization, or refractoriness, the diminution of response observed following chronic exposure to stimulatory drugs or hormones, is a frequently reported but incompletely understood phenomenon. For example, agonist-mediated desensitization of $\alpha$- and $\beta$-adrenergic receptors has been described in many tissues (last reviewed in Refs. 1–3). Numerous reports have emphasized studies of $\beta$-adrenergic receptors and the likelihood that several discrete events are involved in desensitization (1–3). Much less is known regarding desensitization of $\alpha$-adrenergic receptors (4, 5). The changes in adrenergic receptor metabolism that occur during and following agonist-mediated desensitization are poorly defined, and there have been relatively few studies of the regulation of adrenergic receptor metabolism by their natural effectors, epinephrine and norepinephrine (5–8).

We have undertaken studies of the metabolism of adrenergic receptors in a cultured nonfusing muscle cell line, BC3H-1. These clonally derived murine cells express both $\alpha_1$- and $\beta_2$-adrenergic receptors, which have been characterized by both radioligand binding techniques (9–12) and measurement of functional responses (9, 10, 12–15). In addition to the adrenergic receptors, BC3H-1 cells express nicotinic cholinergic and insulin receptors, the metabolism of which has already been described (16–20). Thus, BC3H-1 cells constitute a unique model system for studying the regulation of events mediated by adrenergic agonists acting through two independent receptors. In the present study, we examined several facets of basal and agonist-modified receptor metabolism, in order to define those aspects of receptor metabolism altered following exposure of cells to epinephrine and norepinephrine.

## Experimental Procedures

**Materials.** The following drugs were received as generous gifts: phentolamine mesylate from Ciba-Geigy Corp.; cyanopindolol from Dr. G. Engel, Sandoz Pharmaceuticals; (−)-epinephrine, (−)-isoproterenol, and (−)-norepinephrine as the (+)-bitartrate salts from Sterling-Winthrop Research Institute; (−)-propranolol and (±)-propranolol from

This work was supported by National Institutes of Health Grants GM-31987 and HL 25457.

**ABBREVIATIONS:** POB, phenoxybenzamine; NHNP-NBE, *N*-[2-hydroxy-3-(1-naphthoxy)-propyl]-*N'*-bromoacetylethylenediamine; DME, Dulbecco's modified Eagle's medium; ICYP, iodocyanopindolol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Ayerst Research Laboratories; POB from Smith Kline and French; and NHNP-NBE from Dr. D. Triggle, State University of New York at Buffalo. Phenylephrine and cycloheximide were purchased from Sigma Chemical Co. Carrier-free $Na^{125}I$ (>350 mCi/ml) and [$^3$H]prazosin (81 Ci/mmol) were obtained from New England Nuclear Corp., and [$^3$H] *myo*-inositol (15 Ci/mmol) was from the American Radioligand Co. DME, 4xNF pancreatin, and fetal calf serum were obtained from Grand Island Biological Co. Flasks and dishes were manufactured by Falcon.

**Culture of BC3H-1 muscle cells.** The BC3H-1 nonfusing muscle cells used in this study [described previously by Hughes *et al.* (10)] were a gift from Drs. J. P. Mauger and J. Bockaert, previously located at Collége de France (Paris, France). The cells were routinely grown in Falcon T-flasks in DME supplemented with 10% (v/v) fetal calf serum and maintained at 37° in a humidified atmosphere of 10% $CO_2$ in air. The cells were subcultured at 3-day intervals, using a × 40 dilution of 4xNF pancreatin to dissociate them from the plastic substrate. For experimental purposes, unless indicated otherwise, approximately $5 \times 10^7$ cells were seeded into 150-mm culture dishes containing 25 ml of culture medium. From this starting density, the cells reached confluence within 2–4 days.

**Drug treatment of intact cells.** Cells were grown to confluence on 150-mm dishes. Following incubation with POB and/or NHNP-NBE for 30 min at 37° in DME, the cells were washed four times with 10 ml of DME before being replaced in fresh growth medium. Cycloheximide [1 μg/ml, a concentration which inhibits ≥95% of protein synthesis in these cells (10)] and adrenergic agonists were added directly to the growth medium and the cells were kept in the dark. Oxidative breakdown of the agonists is obviated under these conditions (21).

**Preparation of crude membranes from BC3H-1.** A crude membrane preparation, free of nuclei, was prepared by a modification of a method described previously (10). After reaching confluence (a minimum of 2 days after seeding), the cell sheet was washed twice at 20° with 10 ml of 0.9% NaCl buffered with 20 mM Tris-HCl (pH 7.4) and, using a cell scraper, was collected into 5 ml of the same solution. The cells were collected by centrifugation (400 × *g* for 5 min at 20°) and resuspended in one-fifth the initial volume of 5 mM Tris-HCl (pH 7.6), 1 mM $MgCl_2$ at 4°. After 5 min, during which time visible swelling had occurred as judged by observation under light microscopy, the cells were homogenized at 0–4° by five strokes of a motor-driven "zero-clearance" Potter-Elvehjem homogenizer (Kontes model K-886030). The suspension was centrifuged three times for 2 min at 200 × *g*, each time discarding the pelleted material. The final supernatant, which was substantially free of nuclei and intact cells as judged by microscopic examination, was centrifuged at 38,500 × $g_{av}$ for 10 min. The pellet was resuspended in 20 ml of 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$. The particulate material was washed one additional time and resuspended in the appropriate volume (usually 20–40 ml for six dishes of cells) of the above buffer. This preparation was used immediately in the binding assays and recovers approximately 50% of total cellular receptors, with a 3-fold increase in specific binding activity. Recovery of membrane protein (36–40%) was not altered by state of confluency or by cycloheximide. Of the total cellular receptors, approximately 25% were able to pass through Whatman GF/C filters but were trapped on underlying Whatman GF/B filters soaked in a 1% solution of polyethyleneimine. Thus, 50% of the total receptors were recovered in the membrane fraction which we prepared, 25% were discarded with intact cells and nuclei, and 25% presumably remained in the high speed supernatant.

**Preparation of [$^{125}$I]ICYP.** (±)-Cyanopindolol was iodinated as described previously (10) and stored at −20° for no longer than 2 months, when it still migrated as a single peak on silica gel thin layer chromatography with pyridine/glacial acetic acid/water (0.33:0.6:9.07, v/v) as solvent. The [$^{125}$I]ICYP was assumed to be iodinated to the maximal theoretical specific activity of 2200 Ci/mmol; correction for decay of [$^{125}$I]ICYP assumed that the product of radioactive decay was unable to bind to the β-receptor since comparison with [$^3$H]dihydroalprenolol showed this method to yield a consistent receptor number in rat lung membranes. Fresh [$^{125}$I]ICYP was prepared at least once every 2 months.

**[$^{125}$I]ICYP and [$^3$H]prazosin binding assays.** Membranes prepared from BC3H-1 cells were suspended in 50 mM Tris-HCl (pH 7.6) and 10 mM $MgCl_2$, and were incubated in a final volume of 0.25–1 ml, at a protein concentration of 0.1–0.3 mg/ml. The binding reaction was carried out for 60 min at 30° as previously described (14). Nonspecific binding was always less than 50% in these experiments. Specific and nonspecific binding of both radioligands increased linearly with membrane protein concentrations up to at least 0.24 mg/ml with [$^{125}$I]ICYP and 0.60 mg/ml with [$^3$H]prazosin. In those cases where receptor density was being determined, quadruplicate estimates were made of both total and nonspecific binding at a single radioligand concentration (0.3 or 0.5 nM). ($^3$H]Prazosin samples were counted in a Beckman LS-330 liquid scintillation spectrometer at 40% efficiency; [$^{125}$I]ICYP samples were counted in a Beckman gamma counter at 85% efficiency.

**Measurement of phosphatidylinositol turnover.** Phosphatidylinositol turnover was estimated by a method similar to that described earlier for BC3H-1 cells (15). Cells were seeded into 35-mm dishes and grown until confluent. Following treatment of the cells with epinephrine or irreversible antagonists, the growth medium was aspirated and replaced with 0.75 ml of 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.6 mM $MgSO_4$, 1.0 mM $Na_2HPO_4$, 5.5 mM D-glucose, 25 mM HEPES, pH 7.4, 0.06% (w/v) bovine serum albumin, 4 μCi/ml of [$^3$H]*myo*-inositol, 10 μg/ml of catalase, 10 μg/ml of superoxide dismutase, 0.1 mM (±)-propranolol with or without 10 μM (−)-epinephrine. Following a 30-min incubation at 37°, the medium was aspirated and the cells were washed with two 1-ml aliquots of buffer that contained no radioligand, drugs, or enzymes. The cells were then covered with 1 ml of ice-cold methanol and removed from the plastic substrate with the aid of a cell scraper. The samples were mixed with 1 ml of chloroform and 0.9 ml of 2 M KCl and centrifuged for 15 min at 2000 × *g* to separate the two phases. One ml of the upper phase was reserved for determination of its content of tritium and the remainder of the upper phase was aspirated. The chloroform was washed twice with 1 ml of chloroform/methanol/2 M KCl (3:48:47) before being transferred to scintillation vials and evaporated to dryness. The amount of radiolabel in the chloroform phase was corrected using the amount of radiolabel in the sample of upper phase, assuming proportional recovery of radiolabel in both phases. The hormone-stimulated phosphatidylinositol turnover was estimated as the absolute increase in the chloroform-extractable radiolabel above the mean basal level.

**Measurement of cyclic AMP accumulation.** Cells were seeded into 35-mm dishes and grown until confluent. Following treatment of the cells with epinephrine or irreversible antagonists, the growth medium was aspirated and replaced with 1 ml of DME containing 20 mM HEPES, pH 7.4, 1 mg/ml of bovine serum albumin, 0.2 mM 1-methyl-3-isobutylxanthine, 10 μg/ml of catalase, and 10 μg/ml of superoxide dismutase with or without 1 mM isoproterenol. Following a 10-min incubation at 37°, the medium was aspirated and 1 ml of ethanol containing 1% (v/v) 1 N HCl was added. The cells were sonicated *in situ* and the remaining suspension was subsequently lyophilized to dryness. Cyclic AMP was determined by a competitive binding protein method and the hormonal-responsive increase in cyclic AMP was estimated as the absolute increase in cyclic AMP above the basal mean level.

**Data analysis.** For Scatchard analyses of equilibrium binding data (Figs. 3, 6, and 7), total binding at each concentration of radioligand was determined in triplicate and nonspecific binding in duplicate. Linear regression analyses were carried out for all estimates of nonspecific binding, since these varied linearly over the range of radioligand concentrations tested. Specific binding at each concentration of radioligand was then calculated from the difference between the mean of the values for total binding and the value for nonspecific binding computed from the line given by linear regression. Data were fit to the relationship $B = B_{max}[L]/(K_d + [L])$, which was, in turn, used to simulate the saturation binding isotherms shown in the figures. The

number of binding sites ($B_{max}$) and the dissociation constant ($K_d$) were obtained from Scatchard analyses of the data; [$L$] represents the free radioligand concentration.

In some experiments, single concentrations of radioligand were used to estimate the concentration of receptors. In such cases, 300 pM [$^{125}$I] ICYP or 500 pM [$^3$H]prazosin was used and receptor concentrations were corrected by factors of 1.14× ($\alpha_1$) and 1.33× ($\beta_2$). This correction was calculated from the ratio $(K_d + [L])/[L]$, assuming $K_d$ values of 70 pM for [$^3$H]prazosin ($\alpha_1$) and 100 pM for [$^{125}$I]ICYP ($\beta_2$), from Hughes *et al.* (10).

Time coures of receptor turnover were fit by Marquardt's nonlinear least squares method (22). Thus, receptor loss following treatment of cells with cycloheximide or adrenergic agonist, when assumed to conform to a model of first order decay, was fit to the equation $[R]/[R_o] = \exp(k_c t)$. Receptor recovery following treatment of cells with irreversible antagonists was fit to the equation $[R] = k_s(1-\exp(-k_c t))/k_c$, where $k_s$ is the rate constant of receptor appearance and $k_c$ is the rate constant of receptor clearance.

**Protein determination.** Protein was determined by the method of Peterson (23), employing log-log transformation to yield a linear standard curve (24). Bovine serum albumin, containing sodium azide (1 mg/ml) as a noninterfering bacteriocide, was employed for standards.

## Results

**Regulation of adrenergic receptors during cell growth.** The expression of assayable adrenergic receptors as a function of growth of BC3H-1 cells is shown in Fig. 1. The number of $\beta_2$-receptors, expressed per mg of membrane protein, remained constant throughout logarithmic growth, whereas the number of $\alpha_1$-receptors increased during this period, reaching a stable maximum at confluence. Thus, the ratio of $\alpha_1$- to $\beta_2$-receptors increased during the logarithmic phase of the growth cycle. This indicates disparate rates of appearance and/or clearance of the two receptor subtypes. Receptor "appearance" in these studies includes rates of receptor synthesis as well as subsequent events involved in receptor insertion into the membrane fraction that we used. This membrane fraction is a crude preparation that is probably composed of plasma membrane and other cellular membranes. The term "clearance" refers to the loss of receptors from this membrane fraction. Therefore, in the intact cell, "clearance" represents loss of binding sites through modification of the receptor *in situ*, receptor internalization, and subsequent modification and/or shedding of receptors from the cells.

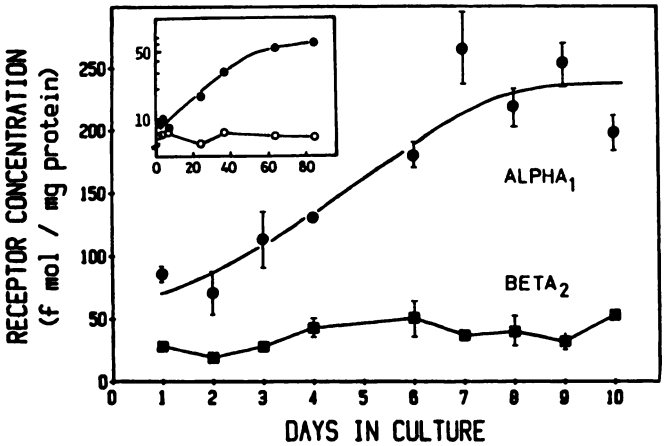


**Fig. 1.** Expression of adrenergic receptors during cell growth. Cells were seeded into 150-mm plates at a lower density than usual ($2\times10^4$ cells/cm$^2$). The growth medium was renewed on day 7. Densities of $\alpha_1$- (●) and $\beta_2$- (■) receptors in membranes prepared from the cells were determined daily; results shown are the mean data from two experiments. The *inset* is a growth curve of BC3H-1 cells seeded at a density of $3\times10^5$ cells/cm$^2$ in the presence (○) or absence (●) of 1 μg/ml of cycloheximide. The *y* axis shows cell density, $10^{-4}$ cells/cm$^2$, and the *x* axis shows hours in culture.

**Assessment of adrenergic receptor metabolism in pre- and post-confluent cultures of BC3H-1 cells by the use of cycloheximide.** In order to assess the turnover rates of the $\alpha_1$- and $\beta_2$-receptors in pre- and post-confluent cultures and to determine whether the difference in $\alpha_1$-receptor expression during cell growth results from changes in appearance and/or clearance, cells at two stages of growth (pre- and post-confluent) were treated with 1 μg/ml of cycloheximide [which inhibits ≥95% protein synthesis in BC3H-1 cells with no effect on cell viability over a period of 4 days (10)]. Since protein and, hence, receptor synthesis has been abolished by this treatment, the change in concentration of receptors (Fig. 2) would be expected to be a measure of the rate constants of receptor clearance and should follow first order kinetics (Table 1). Thus, receptor loss,

$$\frac{d[R]}{dt} = k_c[R]$$

or in integrated form,

$$[R]/[R_o] = e^{-k_c t}$$

where $k_c$ = the rate constant of receptor clearance and $R_o$ = the initial concentration of receptors.

In preconfluent cells, addition of cycloheximide does not elicit first order kinetics of clearance of $\beta_2$-adrenergic receptors from BC3H-1 cells (Fig. 2A). The concentration of $\beta_2$-adrenergic receptors is observed to increase in preconfluent cultures of BC3H-1 cells following treatment with cycloheximide.[1] In postconfluent cultures of BC3H-1 cells, the concentration of $\alpha_1$- and $\beta_2$-adrenergic receptors decay with the expected first order kinetics. The relative stability of the $\beta_2$-receptors *vis-a-vis* the $\alpha_1$-receptors, which turn over far more rapidly, in both pre- and post-confluent cultures is clearly demonstrated (Fig. 2).

**Assessment of adrenergic receptor metabolism in confluent cultures of BC3H-1 cells by the use of irreversible receptor antagonists.** An independent method of assessing the rate of receptor turnover is the use of irreversible antagonists which are specific for each receptor subtype. Cells treated with such irreversible antagonists are unable to bind radiolabeled ligands to their respective receptors. After washing away the irreversible antagonists, one examines the reappearance of specific binding. Since the irreversible antagonists are presumed to penetrate to the cell interior, these binding sites represent "new" receptors whose appearance, as will be shown below, requires protein synthesis. Such antagonists have been described for both $\alpha$- and $\beta$-adrenergic receptors. POB has been used in several systems (25–27), including BC3H-1 cells (11), to study receptor reappearance after irreversible blockade of $\alpha_1$-adrenergic receptors. NHNP-NBE has similarly been used to study $\beta_2$-adrenergic receptors (28, 29).

In order to ascertain that receptor blockade brought about

[1] The concentration of $\beta_2$-receptors in preconfluent cultures appears to rise following treatment with cycloheximide. We find stable expression of total cell protein under this condition (Fig. 1, *inset*) and, thus, we attribute the rise in $\beta_2$-receptor concentration to stable expression of these receptors together with the loss of a fraction of membrane protein.

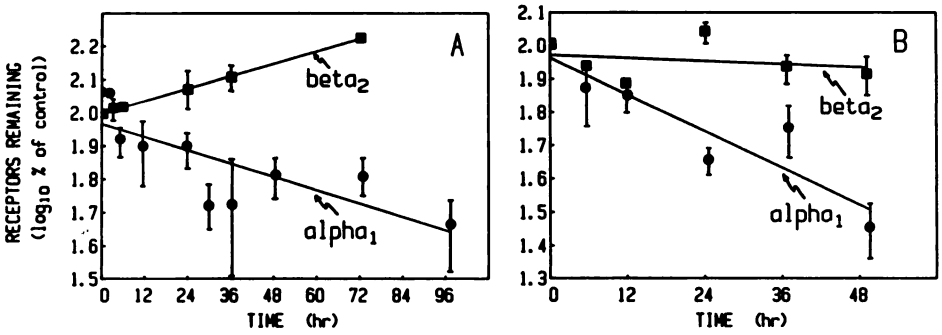


**Fig. 2.** Rates of adrenergic receptor clearance at low and high cell densities. Cells were seeded into 150-mm plates at a low density ($2\times10^4$ cells/cm$^2$). At day 2 (A) or day 8, when the cells were confluent (B), 1 μg/ml of cycloheximide was added to plates. Subsequently, the densities of $\alpha_1$- (●) and $\beta_2$- (■) receptors were measured in membranes prepared from both control and cycloheximide-treated cells at the times indicated. Results are the mean data ± SD from two (A) or three (B) experiments. Control (100%) receptor expression refers to that at time zero, when cycloheximide was added.

TABLE 1

**Rate constants of receptor metabolism under basal conditions**

All data shown here are accrued from Figs. 1, 2, and 3. Rate constants of receptor appearance are in fmol/mg of protein/hr. Rate constants of receptor clearance are in $hr^{-1}$.

| | $\alpha_1$-Adrenergic receptors | | | | $\beta_2$-Adrenergic receptors | | | |
|---|---|---|---|---|---|---|---|---|
| | Preconfluent | | Post-confluent | | Preconfluent | | Post-confluent | |
| | Appearance | Clearance | Appearance | Clearance | Appearance | Clearance | Appearance | Clearance |
| Cycloheximide | >5.3[a] | 0.010 ± 0.002 (69)[b] | 7.8[c] | 0.026 ± 0.004 (27) | 2.2[d] | 0[d] | 0.20[c] | 0.004 ± 0.002 (170) |
| POB | | | 10.2 | 0.034 ± 0.007 (20) | | | | |
| NHNP-NBE | | | | | | | 0.25 | 0.003 ± 0.001 (200) |

[a] A minimum rate constant of $\alpha_1$-receptor appearance in preconfluent cultures was estimated (see Footnote 4).

[b] Numbers in parentheses are estimated receptor half-lives in hours.

[c] These rate constants for receptor appearance were calculated from the product of receptor concentration and the rate constants for receptor clearance, assuming steady state expression of receptors.

[d] The rate constant of $\beta_2$-receptor clearance in preconfluent cells could not be accurately determined in the presence of cycloheximide but was much slower than that of cell protein and was thus assumed to be zero (see Footnote 1). Since the concentration of $\beta_2$-receptors in preconfluent cultures does not change, the rate constant of receptor appearance is the product of the exponential rate constant for increase in cell density (0.043) and the concentration of receptors (50 fmol/mg of protein).

by treating cells with POB or NHNP-NBE was irreversible, it was necessary to confirm that these drugs decreased receptor concentration. Cells were treated with 20 nM POB or 100 nM NHNP-NBE, concentrations which abolished 30–60% of the $\alpha_1$- and $\beta_2$-receptors, as determined by competitive binding studies in membranes using [$^3$H]prazosin or [$^{125}$I]ICYP, respectively. The irreversible antagonists decreased receptor concentration 30% (with little change in the affinity of radiolabeled antagonist) in membranes prepared from these cells (Fig. 3, A and B). However, in measuring the synthesis of new receptors, it is advantageous to start from a lower receptor concentration. Membranes prepared from cells treated with 100 nM POB or 3 μM NHNP-NBE lost >90% of their $\alpha_1$- and $\beta_2$-receptors, respectively. At such low receptor concentrations it was not feasible to determine directly either the number of receptors or the radioligand affinity. Nevertheless, it was possible to determine accurately the effects of POB and NHNP-NBE used at these concentrations since, if such membranes were mixed 1:1 with membranes from control cells, one would expect to measure primarily receptors from control cells. Membranes prepared

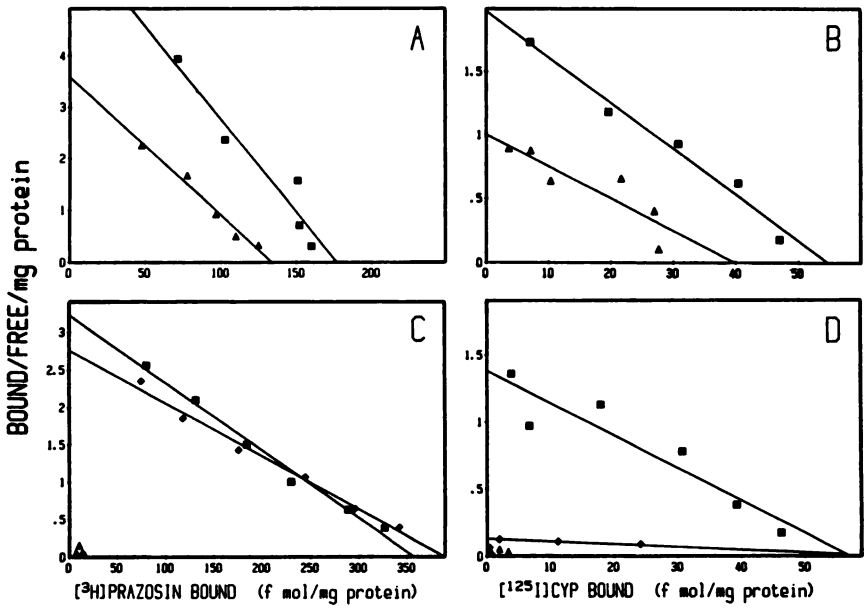


**Fig. 3.** Efficacy of POB and NHNP-NBE in eliciting irreversible receptor blockade. Confluent cells were treated with 20 nM (A) or 100 nM (C) POB, 100 nM (B) or 3 μM (D) NHNP-NBE in DME for 30 min at 37°. Following four washes with 10 ml of DME, membranes were prepared from the cells and saturation binding isotherms were constructed for membranes prepared from control cells (■), cells treated with drug (▲), and, in the case of B and D, a 1:1 mixture of membranes prepared from control and drug-treated cells (◆). In the latter cases, the protein concentrations were considered to be those contributed by membranes prepared from control cells alone.

from cells treated with 100 nM POB lost >90% of their $\alpha_1$-receptors and did not interfere with binding to control membranes when both membranes were incubated together (Fig. 3C). However, in the case of NHNP-NBE, membranes combined from control and treated cells showed a decrease in radioligand affinity (Fig. 3D). This result indicates that 3 $\mu$M NHNP-NBE is partially adsorbed or taken up by the cells and subsequently (after the presumed hydrolysis of the bromoacetyl group) becomes available to compete with the radioligand for receptor-binding sites. Additional experiments, not shown,[2] also indicated that sequestration of drug occurred for two other irreversible $\beta$-receptor antagonists, bromoacetylalprenololmethane (30) and aminobenzyl-propranolol (31). Thus, in BC3H-1 cells, these $\beta_2$-receptor antagonists, NHNP-NBE, bromoacetylalprenolol methane, and aminobenzylpropranolol, have both an irreversible and a competitive component of receptor antagonism.

Confluent cultures of BC3H-1 cells were treated with both 100 nM POB and 3 $\mu$M NHNP-NBE, washed extensively, and returned to growth medium (Fig. 4). Receptor recovery in this situation[3] reflects the appearance of new receptors and their subsequent clearance. Thus, receptor recovery,

$$\frac{d[R]}{d_t} = k_a - k_c[R]$$

or in integrated form,

$$[R] = \frac{k_a}{k_c}(1 - e^{-k_c t})$$

where $k_a$ = the rate constant of receptor appearance and $k_c$ = the rate constant of receptor clearance.

The half-lives of receptors calculated from this method are in the same range as those obtained with cycloheximide and are summarized in Table 1.

**Restoration of receptor function following the application of irreversible receptor antagonists.** Recovery of receptor-stimulated phosphatidylinositol turnover ($\alpha_1$-mediated) and cyclic AMP synthesis ($\beta_2$-mediated) was measured following treatment of confluent cultures of cells with 100 nM POB and 3 $\mu$M NHNP-NBE. The recovery of receptor function parallels the recovery of receptor number, being virtually nonexistent for $\beta_2$-receptors over the course of 8 hr (Fig. 5).

**Summary of the data expressing basal receptor metabolism in BC3H-1 Cells.** The $\beta_2$-receptors appear to be expressed in a stable manner, i.e., they are very slowly cleared from the membrane (Fig. 2, Table 1). In preconfluent cultures of BC3H-1 cells, total cell protein doubles every 16 hr. For the concentration of $\beta_2$-receptors to remain constant, the rate of appearance of these receptors must be equal to the exponential rate constant for increase of cell protein, which is 0.043 $hr^{-1}$. Thus, the rate constant of $\beta_2$-receptor appearance in dividing BC3H-1 cells is approximately 2.2 fmol/mg/hr (Table 1). In contrast, the concentration of $\alpha_1$-receptors in preconfluent cultures increases during the growth of these cells, reaching a stable maximum at confluence. The rate constant for $\alpha_1$-receptor clearance in preconfluent cultures is similar to that observed in post-confluent cultures (Table 1). The rate constant for $\alpha_1$-receptor appearance in preconfluent cultures cannot be precisely determined from the available data.[4]

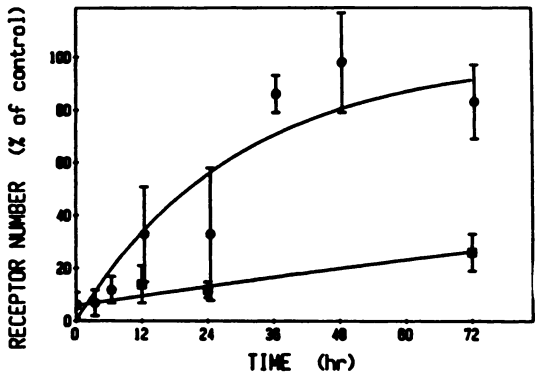


**Fig. 4.** Rate of adrenergic receptor appearance subsequent to treating cells with irreversible receptor antagonists. Confluent cultures of cells were treated simultaneously with 100 nM POB and 3 $\mu$M NHNP-NBE in DME for 30 min at 37°. Following four washes with 10 ml of DME at 37°, fresh growth medium was added to the cells and the rate of appearance of $\alpha_1$- (●) and $\beta_2$- (■) receptors was measured in membranes prepared from control and treated cells. Results are the mean ±SD of three experiments. Control (100%) receptor expression refers to that in untreated cells. This value did not change significantly during the course of this experiment; therefore, an average control receptor expression was used for reference.

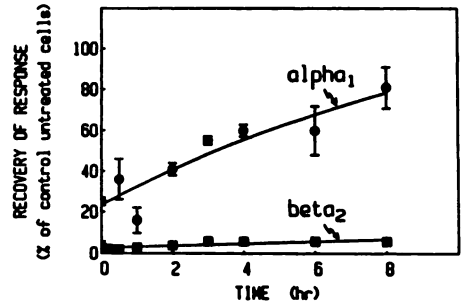


**Fig. 5.** Rate of recovery of adrenergic response subsequent to treating cells with irreversible receptor antagonists. Confluent cultures of cells were treated simultaneously with 100 nM POB and 3 $\mu$M NHNP-NBE in DME for 30 min at 37°. Following four washes with 1 ml of DME at 37°, fresh growth medium was added to the cells and the rate of appearance of $\alpha_1$-receptor-mediated phosphatidylinositol turnover stimulated by 10 $\mu$M (−)-epinephrine (●) and $\beta_2$-receptor-mediated cyclic AMP synthesis stimulated by 1 $\mu$M (−)-isoproterenol (■) was measured. Results are the mean of duplicate determinations. Control (100%) expression of function refers to that in untreated cells.

[2] R. J. Hughes, L. C. Mahan, and P. A. Insel, unpublished results.

[3] Despite the reversible component of receptor ocupation imparted by the use of 3 $\mu$M NHNP-NBE (Fig. 3), we believe that the recovery rate of $\beta_2$-receptors shown in Fig. 4 is a valid estimation. In the experiment in Fig. 3, 3 $\mu$M NHNB-NBE increased the $K_d$ for [$^{125}$I]ICYP 11-fold in membrane preparations. To estimate receptor concentration in Fig. 4, we used 300 pM [$^{125}$I]ICYP. At this concentration and assuming the worst case, we calculate that receptor concentration would be underestimated by 70%. We believe that this is an exaggeration of what occurred in the experiment shown in Fig. 4 because, after NHNP-NBE treatment, washed cells were incubated with 10% serum. We have found that serum facilitates wash-out of hydrophobic antagonists like NHNP-NBE and thus the serum-containing medium would be expected to remove most or all of the NHNP-NBE from the cells. In addition, there was no residual antagonist that would irreversibly occupy receptors following the use of 3 $\mu$M NHNP-NBE since, in the experiment in Fig. 3, there was no effect on the receptor concentration of control membranes when these were mixed with membranes from treated cells.

[4] The rate constant for $\alpha_1$-receptor appearance in preconfluent cultures cannot be precisely determined from the available data. However, it is possible to estimate a minimum value. The concentration of $\alpha_1$-receptors in preconfluent cultures rises during cell growth (>100 fmol/mg of protein on day 3), hence the rate of synthesis must reflect the rate constant of increase in cell protein (0.043 $hr^{-1}$) and the rate constant of receptor clearance (0.010 $hr^{-1}$, Table 1). Thus, the rate constant of $\alpha_1$-receptor synthesis in preconfluent cultures must be greater than 5.3 fmol/mg of protein/hr. We suspect that the rate of receptor appearance changes during growth of the cells to confluence, thereby accounting for the increase in receptor number per mg of membrane protein. However, attempts to define a transition between distinct rate constants for receptor appearance proved unsuccessful.

These data indicate that the rate constants for $\alpha_1$-receptor appearance and disappearance are each similar in pre- and post-confluent cultures of BC3H-1 cells. The half-life of $\alpha_1$-receptors is approximately 20 hr in confluent cultures. In contrast, the $\beta_2$-receptors are considerably more stable, with similar rate constants for clearance in pre- and post-confluent cultures (half-life ≃200 hr). However, we estimate the rate constant of $\beta_2$-receptor appearance to be 10-fold greater in preconfluent compared to post-confluent cultures, suggesting that synthesis of $\beta_2$-receptors almost ceases in confluent cultures.

**Down-regulation of $\alpha_1$- and $\beta_2$-agonist-mediated receptors.** Having defined the metabolism of $\alpha_1$- and $\beta_2$-adrenergic receptors during cell growth to confluence, we studied the influence of adrenergic agonists on receptor expression in confluent cultures of BC3H-1 cells. Incubation of BC3H-1 cells with agonists elicited down-regulation of both $\alpha_1$- and $\beta_2$-receptors. The concentration dependency of agonist-mediated receptor down-regulation was assessed by measuring receptor number in membranes prepared from cells that had been incubated for 16 hr in growth medium containing increasing concentrations of agonists (Table 2). The rank order of potency of the various agonists in eliciting receptor down-regulation was characteristic of the receptors, i.e., in the case of the $\alpha_1$-adrenergic receptors, (−)-epinephrine > (−)-norepinephrine ≥ (−)-phenylephrine ≫ (−)-isoproterenol. For $\beta_2$-adrenergic receptors, (−)-isoproterenol ≥ (−)-epinephrine ≫ (−)-phenylephrine ≥ (−)-norepinephrine. These rank orders of potencies reflect those we previously described in radioligand binding studies in membranes prepared from the BC3H-1 cells (10). However, in general, the agonists were 10- to 100-fold more potent in eliciting receptor down-regulation than they were in competing for receptor occupancy (Table 2). Furthermore, the potency of agonists in eliciting $\beta_2$-receptor down-regulation was far greater than their potency in stimulating cyclic AMP levels, also by a factor of 10–100 (10). It was important to establish that agonist-mediated receptor down-regulation was due to a decrease in measured receptor number and not to a decrease in affinity for radiolabeled antagonists (32). Down-regulation of both $\alpha_1$- and $\beta_2$-receptors induced by a 16-hr incubation of BC3H-1 cells with 1 μM (−)-epinephrine or 1 μM (−)-norepinephrine was due solely to loss of receptor sites (Fig. 6). The maximum extent of down-regulation in all cases was 80–90%.

To ascertain that down-regulation was mediated by occupation of receptors with agonists, we attempted to block the loss of $\alpha_1$- and $\beta_2$-receptors induced by 1 μM (−)-epinephrine with specific antagonists (Fig. 7). Incubation of cells with the $\alpha$-receptor antagonists, phentolamine (10 μM), decreased the affinity of $\alpha_1$-receptors for [$^3$H]prazosin measured subsequently in membranes and also abolished the down-regulation of $\alpha_1$-receptors which occurred during a 16-hr incubation of cells with 1 μM (−)-epinephrine. Phentolamine has a $K_d$ of 7 nM for the BC3H-1 $\alpha_1$-receptors (10), and its incomplete removal from the membranes is presumed to cause the decrease in affinity of $\alpha_1$-receptors for [$^3$H]prazosin. In studies using BC3H-1 membranes, the $\beta$-adrenergic antagonist sotalol was found to have a $K_d$ of 0.56 μM for the $\beta_2$-receptors. The down-regulation of $\beta_2$-receptors during a 3-hr incubation of cells with 1 μM (−)-epinephrine was inhibited 50% by 100 μM sotalol (which has been shown at this concentration to have no effect on receptor density[2]). The incomplete inhibition of $\beta_2$-receptor down-regulation by sotalol is probably explained as follows. Over the course of 3 hr, all of the $\beta_2$-receptors would be expected to bind epinephrine for at least a brief time; for 50% of the receptors, this period of agonist occupation was apparently sufficient to cause loss of the receptor.

The rate of receptor down-regulation was determined in membranes prepared from confluent cells treated with 0.2 and 20 μM (−)-epinephrine or (−)-norepinephrine (Fig. 8). The half-lives of receptors in these settings are shown in Table 3. The maximal rate of down-regulation is greater for $\beta_2$-receptors ($t_{1/2}$ ≃1 hr) than for $\alpha_1$-receptors ($t_{1/2}$ ≃3 hr), as determined with either epinephrine or norepinephrine. There appeared to be some dose dependency of this rate, most notable in the case of the $\beta_2$-adrenergic receptor, for which maximal and intermediate doses of norepinephrine were used (Tables 2 and 3).

The rates of epinephrine-mediated $\alpha_1$- and $\beta_2$-receptor down-regulation were not affected by the presence of 1 μg/ml of cycloheximide (Fig. 9). This observation implies that cycloheximide has no rapid effect on receptor number other than preventing synthesis of new receptors, (cf. Fig. 2). Since blockade of protein synthesis with cycloheximide failed to alter the agonist-induced rates of $\alpha_1$- and $\beta_2$-receptor clearance, down-regulation likely results from increased receptor clearance rather than inhibition of receptor synthesis.

**Rate of receptor recovery following exposure of BC3H-1 cells to agonist.** The rate of receptor recovery following 16 hr exposure of cells to 1 μM (−)-epinephrine is shown in Fig. 10. After a brief (2 hr) lag, receptor number returned rapidly to control values and recovery was essentially complete by 10 hr. The time course for receptor recovery appeared to be identical for both $\alpha_1$- and $\beta_2$-receptors and, in both cases, receptor recovery was abolished in the presence of 1 μg/ml of cycloheximide, implying a requirement for protein synthesis. in additional experiments (not shown), the time course for recovery of $\beta_2$-receptors following a 3-hr incubation of cells with 1 μM (−)-epinephrine was identical to that following a 16-hr incubation.

TABLE 2

**Concentration dependence of adrenergic agonists for occupation of $\alpha_1$- and $\beta_2$-adrenergic receptors and for induction of down-regulation of receptors**

| | $\alpha_1$-Adrenergic receptors | | $\beta_2$-Adrenergic receptors | |
|---|---|---|---|---|
| | Occupation ($K_d$) | Down-regulation ($EC_{50}$) | Occupation ($K_d$) | Down-regulation ($EC_{50}$) |
| (−)-Isoproterenol | >200 | >10 | 0.04, 4 (79%)[a] | 0.0004 |
| (−)-Epinephrine | 2 | 0.03 | 0.09, 10 (63%) | 0.001 |
| (−)-Norepinephrine | 4 | 0.04 | 0.7, 30 (58%) | 0.8 |
| (−)-Phenylephrine | 3 | 0.08 | 5 | 0.2 |

[a] The percentage of receptors in the high affinity form is given in parentheses. All concentrations (μM) represent concentrations calculated to yield 50% receptor occupancy ($K_d$) or 50% down-regulation of receptors ($EC_{50}$). Some of these data are reproduced from Hughes *et al.* (10).

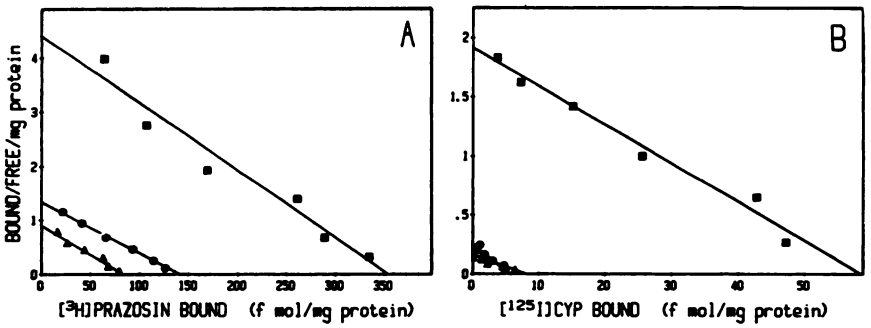


**Fig. 6.** Binding isotherms for down-regulated $\alpha_1$- and $\beta_2$-receptors. Cells were incubated for 16 hr with 1 $\mu$M (−)-epinephrine (▲), 1 $\mu$M (−)-norepinephrine (●) or with no adition (■). Membranes were prepared from each group of cells and saturation binding isotherms of [$^3$H]prazosin (A) and [$^{125}$I]ICYP (B) were constructed. Scatchard plots of these data are shown.

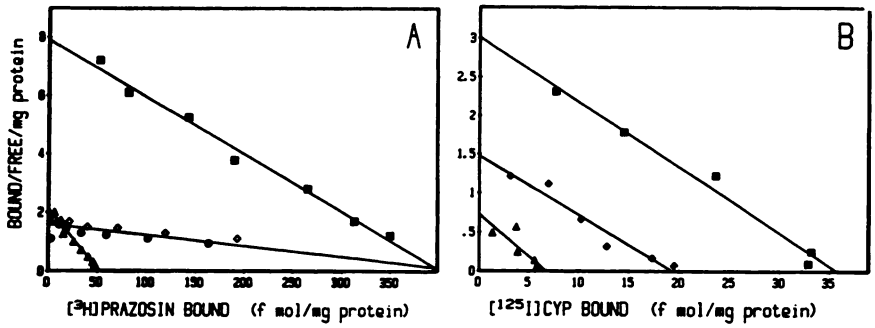


**Fig. 7.** Antagonist blockade of agonist-induced receptor down-regulation. Confluent cells were treated with 1 $\mu$M (−)-epinephrine (▲), 10 $\mu$M phentolamine (●), both drugs (◇), or neither (■) for 16 hr in growth medium (A). Alternatively, cells were treated with 1 $\mu$M (−)-epinephrine (▲), 1 $\mu$M (−)-epinephrine + 100 $\mu$M sotalol (◆), or neither (■) for 3 hr in growth medium (B). Membranes were prepared from each group of cells and saturation binding isotherms of [$^3$H]prazosin or [$^{125}$I]ICYP, respectively, were constructed. Scatchard plots of these data are shown.

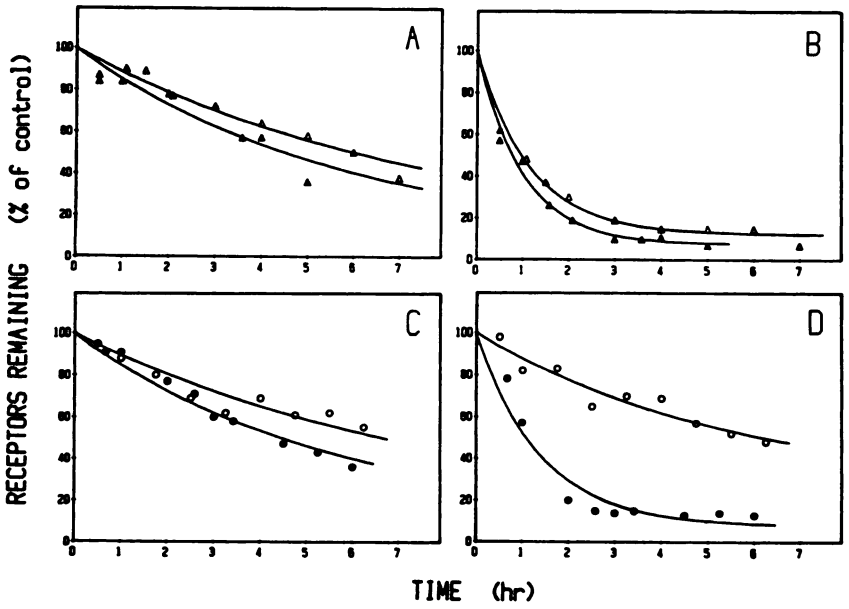


**Fig. 8.** Rate of agonist-induced receptor down-regulation. (−)-Epinephrine at 0.2 $\mu$M (△, ○) or 20 $\mu$M (▲, ●) (△, ▲, A and B) or (−)-norepinephrine (○, ●, C and D) was added directly to the growth medium of confluent cells in the dark. After various times, membranes were prepared and the densities of $\alpha_1$- (C) and $\beta_2$-receptors (B and D) were estimated. These data were fit to the equation $y = 100 + a(e^{-kt}-1)$ using Marquardt's least squares method (28) or, in those curves where a plateau was not reached, by an exponential least squares method where $a$, the maximum percentage of down-regulation, was determined empirically as that value which resulted in the least sum of squares or was fixed at 90%. Control (100%) receptor expression refers to that at time zero when the drugs were added.

TABLE 3

**Rate constants of agonist-mediated receptor down-regulation**

These data were derived from Figs. 8 and 9. The rate constants are expressed ± SE and are in units of hr$^{-1}$.

| | | $\alpha_1$-Adrenergic | $\beta_2$-Adrenergic |
|---|---|---|---|
| (−)-Epinephrine | 0.2 $\mu$M | 0.14 ± 0.02 (5.0)[a] | 0.88 ± 0.07 (0.8) |
| | 1 $\mu$M | 0.13 ± 0.01 (5.5) | 0.75 ± 0.04 (0.9) |
| | 20 $\mu$M | 0.18 ± 0.02 (3.8) | 1.01 ± 0.08 (0.7) |
| (−)-Norepinephrine | 0.2 $\mu$M | 0.34 ± 0.03 (2.0) | 0.18 ± 0.01 (3.9) |
| | 20 $\mu$M | 0.18 ± 0.01 (3.8) | 0.72 ± 0.12 (1.0) |

[a] Numbers in parentheses are estimated receptor half-lives in hours.

**Recovery of receptor function following exposure of BC3H-1 cells to agonist.** The rate of recovery of receptor function ($\alpha_1$: phosphatidylinositol turnover; $\alpha_2$: cyclic AMP synthesis) following 16 hr exposure fo cells to 10 $\mu$M (−)-epinephrine is shown in Fig. 11. Receptor function returns rapidly to control values with no discernible lag. These data suggest that either significant recovery of function occurs during preparation of the cells for assay or function was never completely lost. Restoration of function occurs almost in parallel for $\alpha_1$- and $\beta_2$-receptors, reminiscent of the recovery of receptor number following exposure of cells to agonist (Fig. 10) and in striking contrast to the recovery of receptor number and

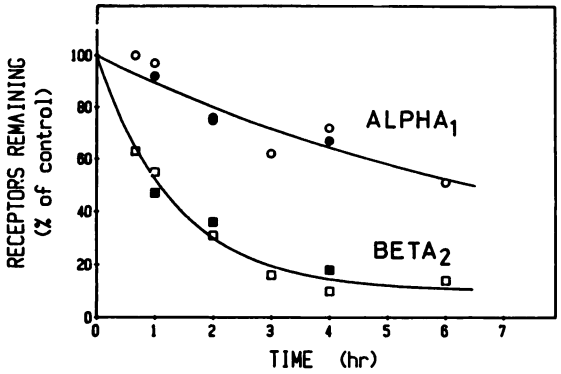


**Fig. 9.** Rate of agonist-mediated receptor down-regulation is not affected by cycloheximide. (−)-Epinephrine (1 μM) was added directly to the growth medium of confluent cells in the dark together with (○, □) or without (●, ■) 1 μg/ml of cycloheximide. After various times, membranes were prepared and the number of $\alpha_1$- (○, ●) and $\beta_2$-receptors (□, ■) was determined. Control (100%) receptor expression refers to that at time zero when the drugs were added.

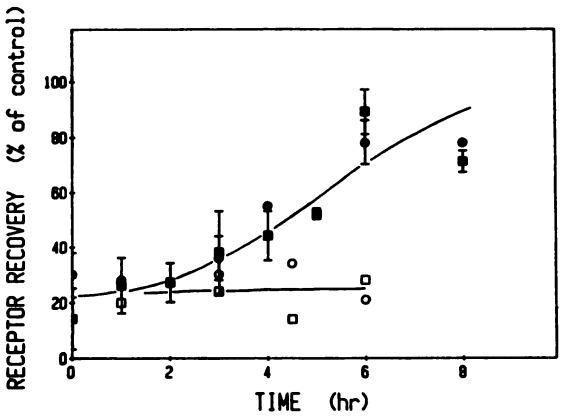


**Fig. 10.** Rate of receptor recovery following exposure to agonist. Confluent cells were incubated for 16 hr in growth medium containing 1 μM (−)-epinephrine. The medium was then aspirated and the epinephrine was washed out four times with 10 ml of DME. Following the addition of fresh growth medium in the presence (○, □) or absence (●, ■) of 1 μg/ml of cycloheximide, membranes were prepared at various times and the densities of $\alpha_1$- (○, ●) and $\beta_2$-receptors (□, ■) were determined. Data are the mean ±SD from three experiments, except for that obtained with cycloheximide (two experiments).

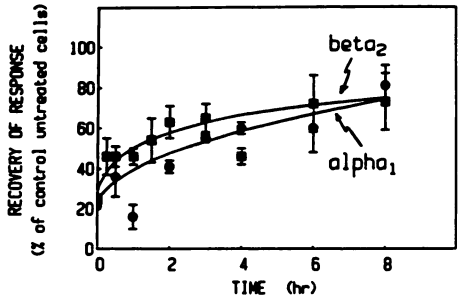


**Fig. 11.** Rate of recovery of adrenergic response subsequent to treating cells with agonist. Confluent cultures of cells were treated with 10 μM (−)-epinephrine for 16 hr in growth medium. Following four washes with DME at 37°, fresh growth medium was added to the cells and the rate of appearance of $\alpha_1$-receptor-mediated phosphatidylinositol turnover stimulated by 10 μM (−)-epinephrine (●) and $\beta_2$-receptor-mediated cyclic AMP synthesis stimulated by 1 μM isoproterenol (■) was measured. Results are the mean of duplicate determinations. Control (100%) expression of function refers to that in untreated cells.

function following treatment of cells with irreversible receptor antagonists (Figs. 4 and 5).

## Discussion

We have investigated the regulation of $\alpha_1$- and $\beta_2$-adrenergic receptor metabolism in the murine muscle cell line, BC3H-1, both during cell growth and in response to agonists. BC3H-1 $\alpha_1$-receptors increase in concentration during the logarithmic phase of cell growth. In addition to $\alpha_1$-adrenergic receptors, insulin receptors (20), nicotinic cholinergic receptors (16, 18), creatine phosphokinase, and myokinase (33) have been reported to increase during the growth to confluence of BC3H-1 cells. Increased expression of these proteins appears to be associated with the spontaneous differentiation of the BC3H-1 cells from myoblasts to non-fusing myocytes. In contrast to the aforementioned receptors, $\beta_2$-receptors are expressed at a constant level during growth of BC3H-1 cells to confluence. It is interesting to note that when the cells become confluent, synthesis of $\beta_2$-receptors appears virtually to cease (Table 1), whereas synthesis of nicotinic cholinergic (16) and presumably also of insulin receptors (20) is initiated. A similar dramatic decrease in the turnover of $\beta$-adrenergic receptors has also been observed when C6 glioma cells reach confluence (8).

Measurement of the rates of $\alpha_1$- and $\beta_2$-receptor appearance and clearance in both pre- and post-confluent cultures of BC3H-1 cells yielded considerably more information on receptor expression. The rates of disappearance of each receptor subtype appear to be constant throughout cell growth but are markedly different for the $\alpha_1$- (~25 hr) and $\beta_2$- (~200 hr) receptors. The two methods that we employed to measure receptor turnover, inhibition of protein synthesis with cycloheximide and recovery of receptors following irreversible blockade, yielded similar estimates for the rates of receptor clearance in confluent cells. Our estimate of the half-life of the $\alpha_1$-receptor in BC3H-1 cells (20–30 hr) is similar to that reported by Mauger *et al.* (11) and also to that of nicotinic cholinergic receptors in these cells [8–16 hr (16, 17, 33)], whereas that of the insulin receptors on BC3H-1 cells was estimated to be 70 hr (19), lying between that of the $\alpha_1$- and $\beta_2$-adrenergic receptors. It should be noted that a comparison of adrenergic receptor half-lives between different systems is difficult, as very disparate rates (varying from 20 hr to several days) have been reported. In contrast, the data presented herein clearly document the independent regulation of two subclasses of adrenergic receptors that are co-expressed on the same cell and that were examined in parallel studies.

There appears to be no detectable intracellular pool of $\alpha_1$-adrenergic receptors awaiting insertion into the plasma membrane since, following treatment of cells with cycloheximide, we found no lag before receptors were lost. This conclusion must be tempered by the possibility that cycloheximide might be blocking the synthesis of a rapidly turning-over protein that is required for receptor insertion. The rate of $\beta_2$-receptor loss was so slow that a lag would not be discernible. In contrast, there is a sizable pool of intracellular nicotinic cholinergic receptors in BC3H-1 cells, amounting to 35% of those present on the cell surface (16), and a smaller but still significant pool of intracellular insulin receptors [~10% of total receptors (19)].

Following a 16-hr incubation of cells with adrenergic agonists, the concentrations of both $\alpha_1$- and $\beta_2$-receptors were

dramatically decreased. The rank orders of potencies of agonists in eliciting this receptor down-regulation were characteristic of the receptor subtypes. However, the concentrations of agonists which effected 50% loss of receptors were far less than those which occupy half the receptors. This discrepancy between agonist concentrations causing receptor occupancy and down-regulation was also observed for insulin receptors in BC3H-1 cells (19) and for β-adrenergic receptors in other cell types (e.g., Ref. 34). The concentrations of agonists which occupy half the receptors ($K_d$ concentrations in radioligand binding experiments) represent "time-average" occupancies. Thus, over a sufficiently long period, rather than half of all the receptors being continually occupied, all the receptors are occupied 50% of the time. Thus, agonists have the potential to down-regulate a greater fraction of receptors than they occupy at any one time. Moreover, it has been suggested that the rate of β-adrenergic receptor loss induced by agonists may relate to the intrinsic activity of these compounds (1, 35).

Receptor down-regulation, even at the highest concentrations of agonist, never entailed complete loss of receptors. There are several possible explanations for this observation. A subpopulation of receptors may exist which is resistant to down-regulation, or receptors may continue to appear but could be cleared at a considerably faster rate, perturbing the steady state to yield a lower receptor density. The rates of receptor down-regulation are substantially faster than the rates of receptor clearance measured under basal conditions with cycloheximide or with recovery of receptors after treating cells with irreversible receptor antagonists. Thus, we conclude that incubation of BC3H-1 cells with adrenergic agonists enhances the rate of both $\alpha_1$- and $\beta_2$-adrenergic receptor clearance, as was reported to be the case for the insulin receptor in BC3H-1 cells (19), the $\alpha_1$- and $\beta_2$-adrenergic receptors in MDCK cells (5), and β-adrenergic receptors in rat kidney cortex (7), L-6 cells (35), and S49 cells (36, 37). Furthermore, whereas the metabolism of $\alpha_1$- and $\beta_2$-receptors is markedly different under resting conditions, the rates of receptor clearance following exposure to agonist are greatly enhanced for both receptor subtypes. The mechanism for this enhanced receptor clearance is conceivably endocytosis, mediated through coated pits and receptosomes/endosomes, in a manner akin to that of other cell surface receptors (38). Support for this conjecture is provided by data demonstrating recovery of β-receptors from agonist-treated cells in "light-vesicles" (1–3) and lysosomes (39). In addition, it has been reported that $\beta_2$-adrenergic receptors in BC3H-1 cells are rapidly internalized following treatment of the cells with isoproterenol, these studies being based upon the rapid loss of specific binding of a hydrophilic antagonist, [$^3$H] CGP-12177, which, under the conditions of the incubation, selectively measures receptors at the cell surface (40).

Following removal of agonist, there was a brief (2 hr) lag before receptors rapidly returned to control levels (10 hr), in a manner which was dependent on protein synthesis. Both $\alpha_1$- and $\beta_2$-receptor subtypes reappeared at the same rate. Protein synthesis-dependent recovery of $\beta_2$-receptors following removal of agonist has also been observed in human astrocytoma cells (in part dependent on whether cells were confluent; Refs. 6 and 41), but not in C6 glioma cells (8). One explanation for such rapid recovery of receptors is that down-regulated receptors are sequestered in an inactive form in the cell, and restoration of their ability to bind adrenergic amines involves protein synthesis. Alternatively, the cell might enhance its rate of receptor synthesis in order to restore receptors rapidly to their initial concentration. We favor the first view, although data in BC3H-1 cells to distinguish the two alternatives are not yet available.

Whether cells are treated with irreversible receptor antagonists or with agonist, the rate of recovery of receptor-mediated function (i.e., phosphatidylinositol turnover and cyclic AMP synthesis for $\alpha_1$- and $\beta_2$-receptors, respectively) is similar to the rate of recovery of receptors. Thus, the rate of recovery of $\beta_2$-receptor-mediated cyclic AMP synthesis following treatment of cells with NHNP-NBE is so slow as to be virtually immeasurable 8 hr after washing out the drug.

In summary, we have demonstrated that $\alpha_1$- and $\beta_2$-adrenergic receptors co-expressed in BC3H-1 muscle cells have very dissimilar basal rates of metabolism, the half-life of the $\alpha_1$-receptor (≃25 hr) being considerably shorter than that of the $\beta_2$-receptor (≃200 hr). Furthermore, the rate of $\beta_2$-receptor appearance declines to almost zero when the cells grow to confluence, whereas the $\alpha_1$-receptors continue to appear at a rate similar to that observed in preconfluent cultures. Following incubation of confluent cultures of BC3H-1 cells with agonists, rapid down-regulation of both $\alpha_1$- and $\beta_2$-receptors occurs through a mechanism independent of protein synthesis. The similar behavior of both receptor subtypes subsequent to agonist exposure suggests that common mechanisms exist for the regulation of receptor expression in response to agonists and that these may be distinct from the normal pathways of receptor turnover.

**Acknowledgments**

We thank S. Dutky for preparation of the manuscript and J. Zappitelli for technical assistance.

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