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Desensitization and Resensitization of β-Adrenergic Receptors in a Smooth Muscle Cell Line

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SUMMARY

Exposure to the β-adrenergic agonist, metaproterenol, elicits extensive receptor loss and desensitization of adenylate cyclase activity in the hamster DDT_1, MF-2 cell line. The reappearance of β-adrenergic receptors and restoration of adenylate cyclase activity were investigated. Receptor reappearance was investigated under conditions in which lost receptors were not detectable either on the cell surface or within the cell. Exposure to metaproterenol resulted in a 3–5-fold decrease in β-adrenergic receptor affinity for agonist, an 85% reduction in β-adrenergic receptor number per cell, and a 65% reduction in isoproterenol-stimulated adenylate cyclase activity without any change in NaF-stimulated enzyme activity. The rate of reappearance of the lost receptors was proportional to the concentration of metaproterenol to which the cells were initially exposed. Metaproterenol, at a concentration of 250 μM, induced long-term receptor loss which required 16 days in fresh media devoid of metaproterenol before the full complement of receptors reappeared. This prolonged receptor loss may be due to residual metaproterenol; however, the resensitization of isoproterenol-stimulated adenylate cyclase activity was restored 2 days after removal of metaproterenol. The lag period for the reappearance of receptors was shortened by incubation with either the β-adrenergic antagonist, nadalol, or the glucocorticoid, methylprednisolone. Both pharmaceuticals reversed receptor down-regulation and up-regulated receptor number in control cells, although the extent and time course of restoration were different. These data suggest that the process of resensitization in DDT_1 cells involves rapid restoration of adenylate cyclase activity and a slower reappearance of receptors over a time period of six population doublings.

INTRODUCTION

Prolonged exposure of intact cells to β-adrenergic agonists leads to a two-step process of catecholamine refractoriness. This process involves rapid uncoupling of β-adrenergic receptors from adenylate cyclase and slower down-regulation of cell surface receptors (1–3). In the former process, occupancy of the receptors by agonists, but not antagonists, leads to a rapid "uncoupling" of the receptors within the plasma membrane. Functionally, there is a decrease in the ability of the receptors to stimulate adenylate cyclase. It can also be seen as a change in the agonist-binding properties of the receptor. The uncoupled receptors are less able to stabilize the high affinity form of the hormone-receptor-nucleotide-regulatory protein complex. As a result, agonist binding to these receptors is of lower affinity and is less responsive to guanine nucleotides. The dissociation constants of the high and low affinity states of the receptor and the ratio of the number of receptors in the high to low affinity states can be determined from computer modeling of the agonist competition curve (4). A decrease in either high affinity binding sites or the ratio of the dissociation constants is consistent with the uncoupled state.

Following receptor uncoupling, there is a loss of cell surface β-adrenergic receptors. The loss and subsequent reappearance of these receptors have been the subject of several recent investigations (5–7). In frog erythrocytes and certain cultured cell lines, β-adrenergic receptors are lost from the cell surface following exposure to isoproterenol. Some studies indicate that only 20% of the "lost" receptors can be identified within the cell (8), while other studies indicate that all of the receptors can be accounted for within the cell (5–7). In these latter studies, when isoproterenol is removed from the bathing media, there is a reappearance of β-adrenergic receptors on the cell surface which does not require new protein synthesis (5, 6, 9). Recent evidence suggests that, with longer exposure

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of cells to isoproterenol, receptors which are lost from the cell surface can no longer be identified within the cell (9, 10). When isoproterenol is removed from these cultures, β-adrenergic receptors reappear within a short time ($t_{1/2}$ = 30 hr), presumably following new receptor synthesis.

To investigate further the reappearance of β-adrenergic receptors on the cell surface following their loss from the cell surface, we assessed receptor number and adenylate cyclase activity in hamster DDT_1 MF-2 (DDT_1) cells after prolonged exposure to the β-adrenergic agonist, metaproterenol. The reappearance was investigated under conditions in which lost receptors were no longer detectable either on the cell surface or within the cell.

MATERIALS AND METHODS

Cell culture. DDT_1 cells, a smooth muscle cell line derived from a leiomyosarcoma of the ductus deferens of a Syrian hamster, were obtained from Drs. J. S. Norris and L. E. Cornett, University of Arkansas, (Little Rock, AK) (11). Monolayer cultures of DDT_1 cells were grown under 5% CO_2 in 75-cm^2 flasks in Dulbecco's modified Eagle's medium containing 4 mM glutamine, 4.5 g of glucose/liter, 2.5% horse serum, 2.5% bovine serum, and 1% gentamicin sulfate (Microbiological Associates, Los Angeles, CA) from an initial inoculum of 8 × 10^4 cells/ml. Cells formed confluent monolayers in 4 days.

Incubation with drugs. Cells were allowed to grow for 24 hr after an initial inoculum of 2.5 × 10^5 cells/ml. At this time (day 0), the β-adrenergic agonist, metaproterenol (1–250 μM), was added to the culture medium and cells were allowed to grow an additional 24 hr. Metaproterenol was removed on day 1 by harvesting the cells with the aid of a rubber policeman, washing two times by centrifugation at 600 × *g* for 15 min, and reinoculating in fresh media. Cells in parallel flasks for control and other drug additions were also harvested and reinoculated in fresh media. To some flasks, at day 1, either nadalol (1 μM) or methylprednisolone (1 μM) was added. At day 2, some flasks were harvested as described below while the remainder were left undisturbed. At day 3, all cells were harvested and one portion was reinoculated in fresh media at a 10:1 dilution and the remainder used for β-adrenergic evaluation. This process was repeated every fourth day. The addition of a pharmacological agent did not alter the growth rate of the DDT_1 cells.

Cell harvesting. Monolayer cells were harvested with the aid of a rubber policeman, centrifuged at 600 × *g*, and lysed at 4° in 5 mM Tris, pH 7.4, for 15 min. Lysates were assayed for β-adrenergic receptors and agonist affinity. Membranes were prepared from some lysates by centrifugation at 48,000 × g and resuspended in 18 mM $MgCl_2$, 0.08 mM ascorbic acid, and 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4. Membranes were assayed for adenylate cyclase activity, β-adrenergic receptor number, and agonist affinity.

Binding assays. β-Adrenergic receptor density was determined by eight-point Scatchard analysis of [^{125}I]ICYP[2] (New England Nuclear, Boston, MA; specific activity 2200 Ci/mmol) binding. Briefly, aliquots of lysate or membrane derived from 2 × 10^5 cells and 5–200 pM [^{125}I] ICYP were incubated with and without 1 μM propranolol for 90 min at 37° in a total volume of 250 μl. For lysates, incubations were terminated by immersion in an ice bath and addition of 100 μg of bovine γ-globulin and 350 μl of 18% polyethylene glycol. After 15 min, tubes were diluted with 2 ml of buffer used for resuspension, and bound [^{125}I]ICYP was separated by a filtration assay (12). For membranes, incubations were terminated by dilution and bound [^{125}I]ICYP was separated by a filtration assay. Nonspecific binding in both lysate and receptor assay was less than 5%. Membrane β-adrenergic receptor affinity for agonist was determined from Hill plots of [^{125}I]ICYP (30 pM) competition with metaproterenol for 90 min, or with isoproterenol for either 5 min (initial velocity) or 90 min (equilibrium). Equilibrium competition curves were resolved by a nonlinear least squares computer modeling method into high and low affinity binding sites of the receptor with the LIGAND (Applesoft) program (Biomedical Computing Technology Information Center, Vanderbilt University, Nashville, TN).

Enzyme assay. Adenylate cyclase activity was determined as previously described (13).

Statistics. Data were analyzed by one-way ANOVA (14).

[2] The abbreviations used are: ICYP, iodocyanopindolol; ANOVA, analysis of variance.

RESULTS

Scatchard analysis of [^{125}I]ICYP binding to DDT_1 lysates yielded a straight line consistent with a single class of saturable antagonist binding sites (Fig. 1, *top*). There were 21,337 ± 729 (n = 7) receptors per cell with an antagonist dissociation constant of 17.2 ± 0.2 pM. [^{125}I]ICYP binding was specific and stereospecific (Fig.

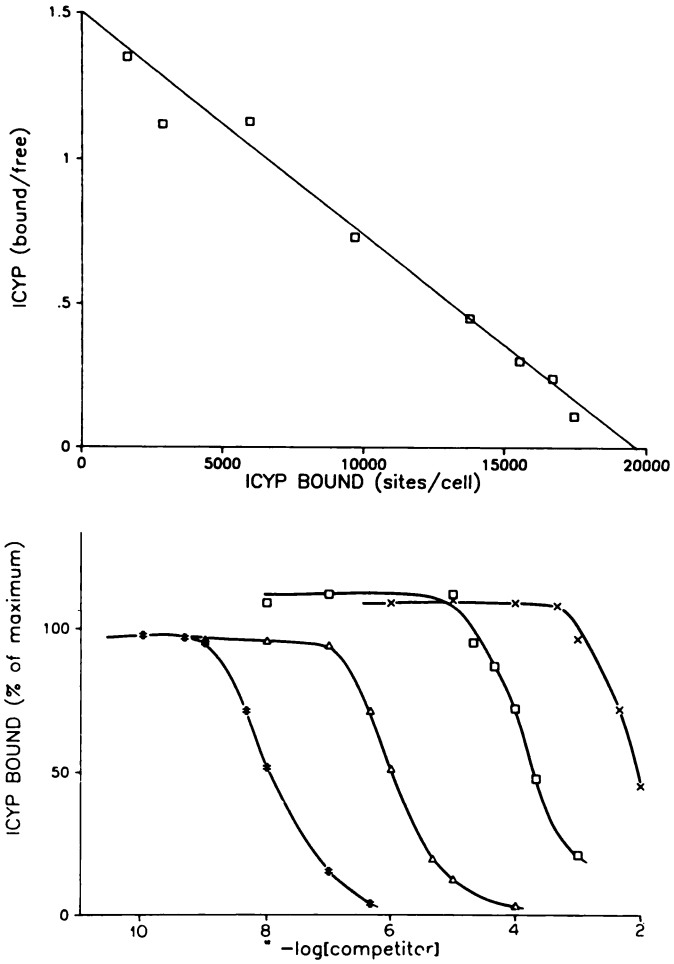

FIG. 1. *Scatchard analysis of [^{125}I]ICYP binding to DDT_1 cell lysate from a single experiment (top) and competition of unlabeled agonists and antagonists for [^{125}I]ICYP-binding sites (bottom)*

Top: receptor density, indicated by the abscissa intercept, was 19,660 sites/cell, and the antagonist dissociation constant, indicated by the negative reciprocal of the slope of the line, was 1.81 × 10^{-11} M. The line was determined by regression analysis with correlation coefficient of 0.99. Bottom: competition with (−)-propranolol (#) with an apparent K_d of 7.4 × 10^{-10} M is 1,000 times more potent than that with (+)-propranolol (△), with a K_d of 8.5 × 10^{-7} M. Competition with epinephrine (□), with a K_d of 8.5 × 10^{-5}, is 28 times more potent than with norepinephrine (×), with a K_d of 2.4 × 10^{-3} M.

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1, *bottom*). Competition with (−)-propranolol was three orders of magnitude more potent that with (+)-propranolol. Competition with epinephrine was 28 times more potent than with norepinephrine, consistent with β_2-type adrenergic receptors (Fig. 1, *bottom*).

Receptor Down-Regulation and Reappearance

In order to investigate the reappearance of β-adrenergic receptors under conditions in which lost receptors were not detectable either on the cell surface or within the cell, DDT_1 cells were exposed to 250 μM metaproterenol for 24 hr. Following exposure to metaproterenol, β-adrenergic receptor number, which was determined in both membranes and whole cell lysate preparations, decreased to less than 15% of untreated cells (Fig. 2, *top*). Under these conditions, there was no difference between whole cell (cell lysate) or cell surface (membrane) receptor number.

The reappearance of receptors was investigated in DDT_1 cell lysates after removal of metaproterenol. Receptor number per cell remained at a reduced level through day 7, with partial recovery at day 14 and full recovery at day 17. When fresh mateproterenol was added at each reinoculation, no recovery in receptors per cell was observed (Fig. 2, *top*). In these experiments, cells were reinoculated in fresh media at day 1 (when the metaproterenol was removed) and at days 3, 7, 11, and 14. In some experiments, an additional two washes (for a total of four) were performed at day 1 and receptor number was monitored for the next 3 days. These additional washes had no effect on the reappearance of β-adrenergic receptors.

In these cells, metaproterenol is a full, but less potent, β-adrenergic agonist than isoproterenol. Metaproterenol is 40–120 times less potent than isoproterenol in the ability to stimulate adenylate cyclase and to compete for β-adrenergic binding sites (dissociation constant) (Fig. 3). Metaproterenol at 250 μM represents a concentration that is approximately 120 times the receptor dissociation constant (Fig. 3, *top*) and 40 times the adenylate cyclase activation constant (Fig. 3, *bottom*) of isoproterenol. We then investigated the ability to recover from receptor down-regulation induced by lower doses of metaproterenol.

Each concentration of metaproterenol between 1 and 250 μM induced equivalent receptor loss; however, the extent of reappearance of receptors at 1 and 2 days after removal of metaproterenol was a function of the initial concentration of metaproterenol present between day 0 and day 1 (Fig. 2, *bottom*). At day 3, there was no recovery following incubation with 200 or 100 μM metaproterenol; however, there was some recovery following incubation with 10 μM and a 50% reappearance of receptors following incubation with 1 μM metaproterenol (Fig. 2, *bottom*).

Effects of a β-antagonist on receptor reappearance. Attempts were made to enhance the rate of recovery from metaproterenol-induced down-regulation. The β-adrenergic antagonist, nadalol (1 μM) was added at day 1 to the media of both control flasks and metaproterenol-treated flasks after removal of the metaproterenol. In the presence of nadalol, receptors recovered rapidly and were

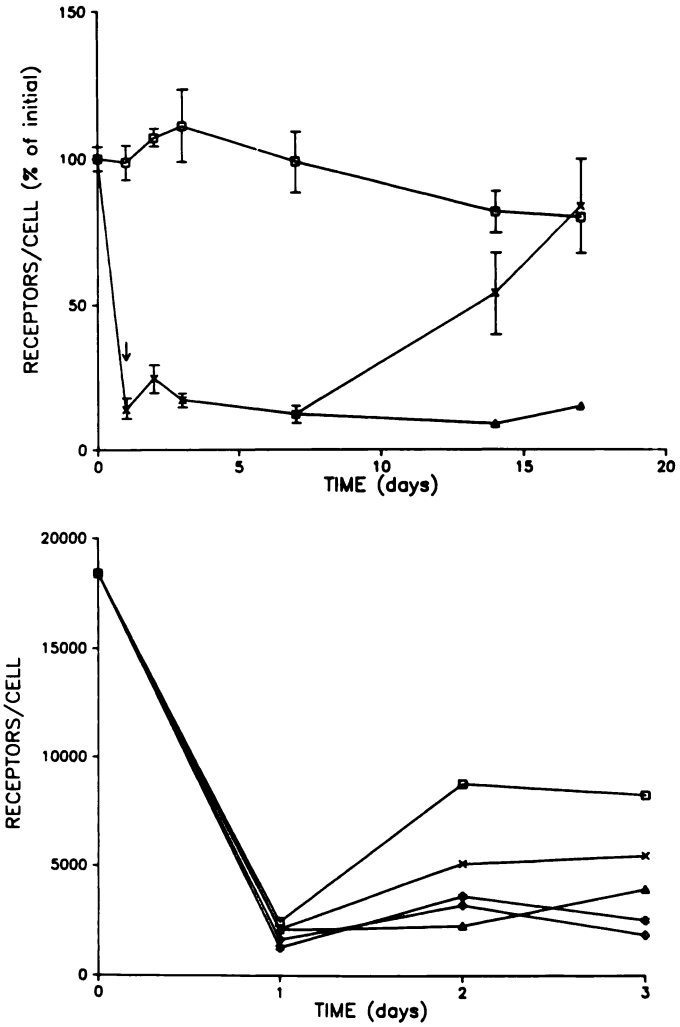

FIG. 2. *Time course of loss and reappearance of β-adrenergic receptors per cell after incubation with metaproterenol*

Metaproterenol was present in culture media for 24 hr between days 0 and 1. There were no differences in cell growth rate or viability with β-agonist treatment. The arrow (top) indicates time of agonist removal when cells were washed twice and reinoculated in fresh media. Initial receptor number was 21,337 ± 729 (n = 7). Top: time course of reappearance of β-adrenergic receptor number per cell after removal (↓) of 250 μM metaproterenol (×) compared to untreated cells (□) and cells in which the metaproterenol was not removed (△). Every 3–5 days at 8×10^5 cells/ml, cells were harvested and reinoculated at 8×10^4 cells/ml. Data represent the mean ± standard error of 4–5 experiments. Bottom: time course of loss and reappearance of β-adrenergic receptor number per cell after 1 day of incubation with 1 μM (□), 10 μM (×), 50 μm (△), 100 μM (#) and 250 μM (◇) metaproterenol. The agonist was removed at day 1, then cells were washed twice and reinoculated in fresh media. Data represent one of three experiments.

indistinguishable from control cells after 1 day (Fig. 4, *top*). Nadalol, when added to control cells, induced receptor up-regulation which was apparent 1 day after addition of the antagonist. Six days after the addition of nadalol (at day 7), receptors per cell increased 2-fold in comparison to control flasks. Similarly, in the metaproterenol followed by nadalol-treated flasks, receptors per cell were nearly doubled at day 7 compared to control flasks (Fig. 4, *top*).

The increase in receptor number after incubation with

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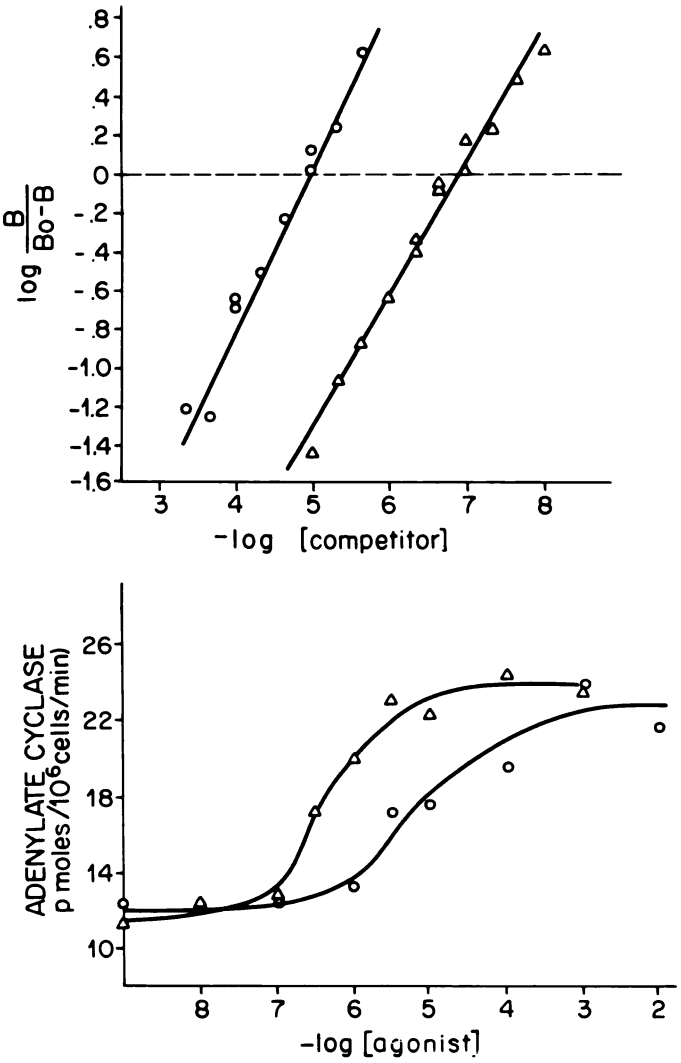

FIG. 3. *Hill plots constructed from competition of [^{125}I]ICYP with isoproterenol (△) or metaproterenol (○) (top), and isoproterenol- (△) and metaproterenol (○)-stimulated adenylate cyclase activity in DDT$_1$ cell membrane preparations*

Top: lines were determined by regression analysis. Hill coefficients (indicated by the slope of the line) are 0.7 and 0.8; competition concentrations at 50% competition (indicated by the dashed *line* intercept) are 1.1×10^{-7} M and 1.1×10^{-5} M, and calculated dissociation constants are 2.5×10^{-8} M and 2.1×10^{-6} M for isoproterenol and metaproterenol, respectively. Bottom: the ordinate represents activity in the presence of agonist plus GTP (1×10^{-4} M) minus the activity in the presence of GTP. The agonist concentrations at 50% maximum activity are 3.5×10^{-7} M and 6.3×10^{-6} M, respectively, for isoproterenol and metaproterenol.

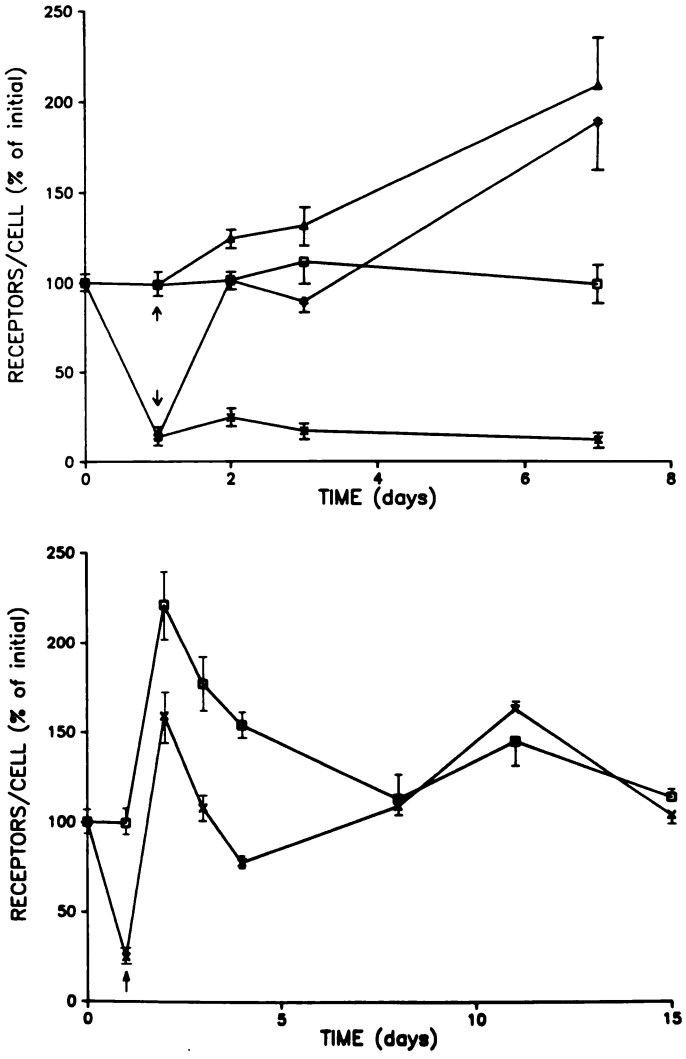

FIG. 4. *Time course alteration in β-adrenergic receptor number per cell following incubation with metaproterenol or nadalol (top) or following a preincubation with nadolol (bottom)*

Top: some cells were incubated with 250 μM metaproterenol for 1 day and washed twice (↓). Cells were reinoculated with no addition (×) or with 1 μM nadalol (#). Nadalol (△) was also added to control (□) flasks at day 1 (↑). Initial receptor number was 22,539 ± 207 receptors/cell. Data represent the mean ± standard error of 4–5 experiments. $p < 0.001$ for difference between control and metaproterenol-treated cells at all days. $p < 0.01$ (nadalol, △) and $p < 0.05$ (nadalol, #) for difference from control cells at day 7 by one-way ANOVA. There were no differences in cell growth rate with nadalol treatment. Bottom: cells were incubated for 4 days with 1 μM nadalol (□), at which time 2 mM metaproterenol (×) was added to some flasks (day 0). At day 1, all cells were washed two times, incubated in fresh media for 1 hr, rewashed, and either harvested or incubated for additional days in the appropriate media. Initial receptor number was 20,475 ± 1449 receptors/cell. Data represent the mean ± standard error of three experiments. $p < 0.001$ (days 1 and 4) and $p < 0.025$ (days 2 and 3) for differences between means by one-way ANOVA.

nadalol suggests that the DDT$_1$ cells are already down-regulated due to the presence of catecholamines. Heat-inactivated horse and bovine sera were assessed for catecholamines by high-performance liquid chromatography (HPLC) and were found to contain 170 and 140 pg/ml of norepinephrine and 900 and 1030 pg/ml of epinephrine, respectively. The final catecholamine concentration in the culture media was 3.3×10^{-10} M.

In the following experiment, the presence of serum catecholamines were negated by preincubation for 4 days with 1 μM nadalol. At this time, metaproterenol (2 mM) was added to some flasks (in addition to 1 μM nadalol) and receptor number was assessed. Receptors per cell decreased to 25% of the control (nadalol-treated) cells (Fig. 4, *bottom*). After 1 day, the metaproterenol was removed (but nadalol retained) as described in the legend to Fig. 4 (*bottom*) and receptor reappearance followed. There was an immediate reappearance of receptors 1 day

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after removal of the metaproterenol, but receptors per cell remained less than in control cells. Control and metaproterenol flasks were indistinguishable 7 days after the removal of metaproterenol.

Effects of a glucocorticoid on receptor reappearance. The recovery from metaproterenol-induced down-regulation was also enhanced by the addition of the glucocorticoid, methylprednisolone, at 1 μM. In a manner similar to that of the above experiments, methylprednisolone was added at day 1 to control flasks and metaproterenol-treated flasks after removal of the metaproterenol. In the presence of methylprednisolone, down-regulated receptors reappeared over a 6-day period compared to 17 days in the absence of the glucocorticoid (Fig. 5). In control cells, methylprednisolone doubled the number of β-adrenergic receptors after 2 days (Fig. 5).

Receptor Agonist Affinity

The isoproterenol dissociation constant was determined for receptors which remained on the cell surface following metaproterenol exposure. Hill plots of initial velocity experiments indicated a 5-fold increase in the isoproterenol dissociation constant of metaproterenol-treated compared to untreated cells (Table 1). Hill plots derived from equilibrium binding data indicate a 3-fold increase in the dissociation constant in metaproterenol-treated compared to untreated cells (Table 1). The magnitude of the increase in the receptor-isoproterenol dissociation constant was proportional to the concentration of metaproterenol in the range from 1–250 μM (Fig. 6). The increase in the dissociation constant (decreased affinity) suggests a decrease in the ability to stabilize the high affinity binding complex. The equilibrium binding data were subjected to two-site computer analysis. The percentage of receptors in the high affinity binding state decreased from 63.4% to 22.8% in untreated compared to metaproterenol-treated cells (Table 1).

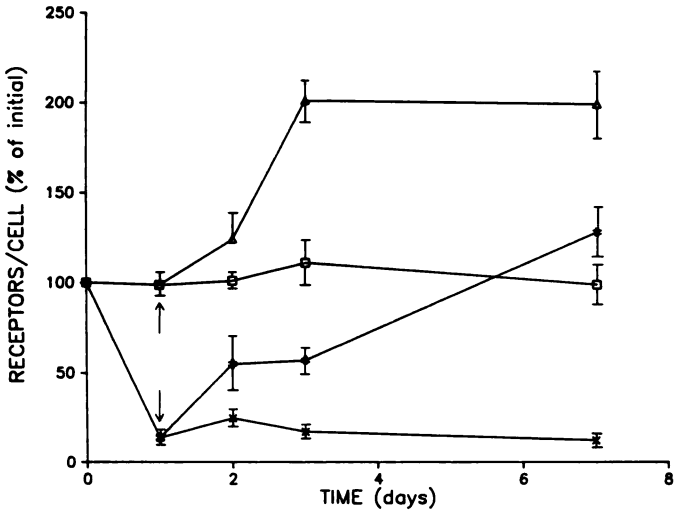

FIG. 5. *Time course alteration in β-adrenergic receptor number per cell following incubation with methylprednisolone*

Conditions were identical to those in Fig. 4, *top*, except that at day 1 (*arrows*), 1 μM methylprednisolone (#, △) was added to one-half the control (□) flasks and one-half the metaproterenol (×) flasks. Initial receptor number was 21,606 ± 1046 receptors/cell. Methylprednisolone had no effect on cell growth rate. Data represent the mean of 4–5 experiments. $p < 0.001$ (days 3 and 4) for difference between methylprednisolone and metaproterenol flasks. $p < 0.01$ (day 3) and $p < 0.05$ (day 7) for difference between methylprednisolone and control flasks by one-way ANOVA.

TABLE 1

Isoproterenol dissociation constants following exposure to metaproterenol

Dissociation constants were determined in membrane preparations from isoproterenol competition with [^{125}I]ICYP (Hill plots) for either 5 min (initial velocity) or 90 min (equilibrium) in untreated and metaproterenol-treated cells. Membranes were prepared from cells harvested 0–48 hr after removal of metaproterenol. Equilibrium binding data were subjected to two-site computer analysis to determine the percentage of receptors in the high affinity binding state. Data represent the mean ± standard error of six determinations.

	Dissociation constant (10^{-8} M)		Receptors in high affinity state
	Initial velocity	Equilibrium	
			%
Control	1.04 ± 0.5	8.24 ± 0.19	63.4 ± 6.5
Metaproterenol	5.57 ± 1.65	22.1 ± 8.9	22.8 ± 9.2
p value	0.024	0.202	0.001

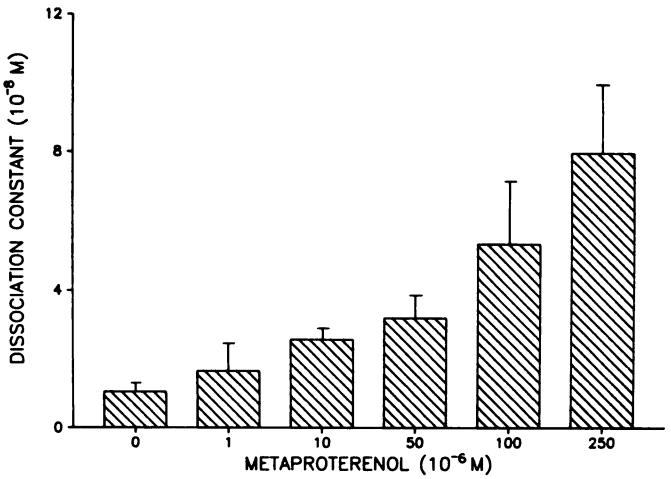

FIG. 6. *Following 1 day of incubation with various concentrations of metaproterenol (1–250 μM), receptor number decreases to 12% of control cells (day 1, Fig. 2, bottom)*

Agonist dissociation constants were determined for the remaining receptors from isoproterenol competition with [^{125}I]ICYP (Hill plots) after 1 day of incubation at the indicated metaproterenol concentrations. Dissociation constants were calculated from the concentration at 50% competition and at an ICYP concentration of 30 pM. Data represent the mean ± standard error of three determinations. $p < 0.02$ for difference in dissociation constant with drug treatment by one-way ANOVA.

Adenylate Cyclase Activity

NaF- and isoproterenol-stimulated adenylate cyclase activities were assessed in membranes following incubation with metaproterenol and/or nadalol. NaF-stimulated adenylate cyclase activity was unchanged from control under all conditions investigated (83.72 ± 3.10 pmol of cAMP/10^6 cells/min). Following incubation with 250 μM metaproterenol, there was a reduction in the isoproterenol-stimulated adenylate cyclase activity to 35% of untreated cells. After removal of the metaproter-

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enol, this desensitization to isoproterenol partially recovered by day 2 and fully recovered by day 3 (Fig. 7). This is in contrast to the 17-day recovery period before complete reappearance of β-adrenergic receptors following down-regulation (Fig. 2, *top*). When nadalol was added to the culture media after the removal of metaproterenol, full responsiveness to isoproterenol was observed after just 1 day. When nadalol was added to the media of control cells, there was no effect on isoproterenol-stimulated adenylate cyclase activity (Fig. 7).

DISCUSSION

We have identified [^{125}I]ICYP-binding sites with the characteristics of a β_2-type adrenergic receptor on DDT_1 cells. Specific binding of [^{125}I]ICYP to DDT_1 membranes yields linear Scatchard plots, indicating a single class of saturable antagonist binding sites. Competition with (−)-propranolol was 3 logs more potent than that with (+)-propranolol, demonstrating stereospecificity; and competition with epinephrine was 30 times more potent than competition with norepinephrine, consistent with β_2-adrenergic receptors.

In several different cell types, agonist-induced refractoriness of β-adrenergic receptors involves the following characteristics (1–10). First, the receptor is uncoupled from adenylate cyclase. This is characterized by a reduced affinity of the receptor for β-adrenergic agonists and reduced β-adrenergic-stimulated adenylate cyclase activity (homologous desensitization). Sometimes there is a concomitant reduction in GTP-, NaF-, or other hormone-stimulated enzyme activity (heterologous desensitization). Second, 50–90% of the β-adrenergic receptors are not detectable on the cell surface but are identifiable within the cell. Third, continued exposure to β-adrenergic agonists results in a loss of receptors which are no longer detectable either on the cell surface or within the cell. Finally, after removal of the β-adrenergic agonist, the receptors reappear on the cell surface.

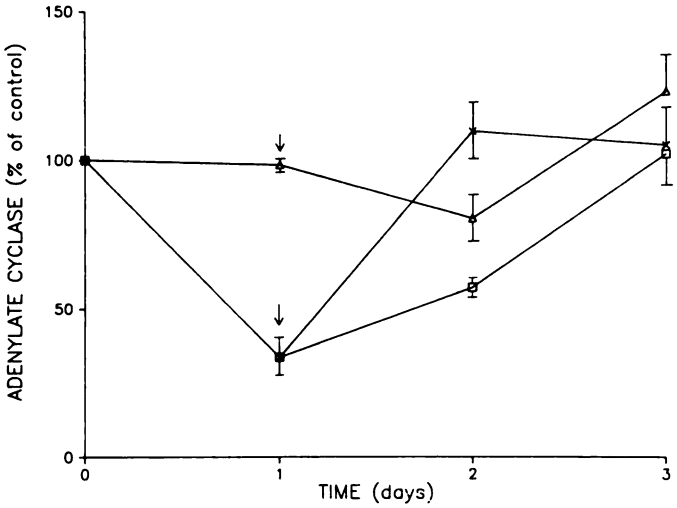

FIG. 7. *Time course of desensitization and resensitization of isoproterenol-stimulated adenylate cyclase activity*

Cell culture conditions were identical to those described in Fig. 4, *top*. The addition of nadalol (△) to control cells had no effect on isoproterenol responsiveness. Metaproterenol (□) induced a desensitization which required 2 days for resensitization or 1 day in the presence of nadalol (×). The ordinate represents the percentage of activity in the presence of isoproterenol (1×10^{-4} M) and GTP (1×10^{-4} M) compared to control cells. Total activity in the presence of isoproterenol and GTP was 46.1 ± 11.2 pmol of cAMP/10^6 cells/min, which represents 1.54 times the activity in the presence of GTP alone. Data represent the mean ± standard error of 4–5 experiments. $p < 0.001$ (day 1) and $p < 0.01$ (day 2) for differences from control cells by one-way ANOVA.

Agonist-induced refractoriness in DDT_1 cells appears to involve a similar process. Exposure to the β-adrenergic agonist, metaproterenol, leads to homologous desensitization, that is, a 3–5-fold decrease in β-adrenergic agonist affinity, an 85% reduction in β-adrenergic receptor number, and a 65% reduction in isoproterenol-stimulated adenylate cyclase activity without any change in NaF-stimulated enzyme activity.

The reduction in agonist affinity is apparent from both equilibrium and initial velocity experiments. The conditions for equilibrium agonist competition (90 min, 37°), themselves, may induce decreases in receptor agonist affinity. Initial velocity competition experiments have been shown to be more appropriate for assessment of receptor agonist affinity (5). The 5-fold decrease in receptor agonist affinity and the increase in the number of receptors in the low affinity state indicate that the β-adrenergic receptors are uncoupled following metaproterenol treatment.

After a 24-hr exposure to metaproterenol, β-adrenergic receptors were not detectable either on the cell surface or within the cell. The rate of reappearance of these lost receptors was proportional to the concentration of metaproterenol to which the cells were exposed. Following removal of 1 μM metaproterenol from the culture media, there was a rapid reappearance of 50% of the lost receptors in 1 day.

The half-life of β-adrenergic receptors has been reported to be 25–30 hr and the rate of synthesis is approximately 2%/hr (15). The reappearance of receptors in this study following removal of 1 μM metaproterenol is consistent with these values.

However, when cells were incubated with 250 μM metaproterenol, there was persistent receptor loss. If metaproterenol was not removed from the culture media, no reappearance of receptors was observed. When metaproterenol was removed after 1 day, there was no reappearance for the next 7 days and complete recovery required 17 days. This lag period for the reappearance of receptors was shortened by incubation with either the β-adrenergic antagonist, nadalol, or the glucocorticoid, methylprednisolone. Nadalol rapidly reversed receptor loss with the complete complement of receptors being detectable 1 day after incubation with the antagonist. Nadalol may be acting by blocking the effects of any residual metaproterenol, by an up-regulation of receptors, or by a combination of both. Nadalol, when incubated with control cells, up-regulates receptor number to 200% of untreated cells. Cells exposed to metaproterenol, then nadalol, also demonstrate twice the number of β-adrenergic receptors at day 7 compared to untreated cells. These data suggest that nadalol up-regulates receptor number by reversal of receptor down-regulation from

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catecholamines present in the sera or added metaproterenol. Residual metaproterenol may be a factor in prolonged receptor loss; however, receptor number in metaproterenol-treated cells was repeatedly less than that of control cells for at least 7 days despite multiple washes, a wash with a 60-min incubation to reverse bound metaproterenol (Fig. 7), or the addition of nadalol (Figs. 4 and 7). These data and the observation of a different time course for the reappearance of isoproterenol-stimulated adenylate cyclase activity do not support this possibility.

We and others have previously demonstrated that glucocorticoids increase β-adrenergic receptor number both in the basal and down-regulated states (13, 15, 16). Similarly, in the present studies, methylprednisolone reversed the persistent receptor down-regulation induced by metaproterenol in DDT_1 cells. Glucocorticoids have been shown to increase the rate of new β-adrenergic receptor synthesis (15). This may represent the mechanism of methylprednisolone reversal of β-adrenergic down-regulation observed in the present study. The reversal by methylprednisolone was slower than that of nadalol, suggesting that the mechanisms may be different.

Desensitization of isoproterenol-stimulated adenylate cyclase was observed after 1 day of incubation with metaproterenol. After removal of metaproterenol, resensitization occurred within 1 day in the presence and 2 days in the absence of nadalol. This is in contrast to the 17 days required for complete reappearance of receptors. The time course of desensitization involves a rapid loss of adenylate cyclase activity and a slower down-regulation of receptors (1–3). In some systems, it has been observed that low concentrations of agonists can lead to desensitization of adenylate cyclase without concomitant changes in receptor number (17). Our data suggest that resensitization in DDT_1 cells involves a rapid return of isoproterenol-stimulated adenylate cyclase activity and slow reappearance of receptor number.

Collectively, our data indicate that metaproterenol induces a prolonged β-adrenergic receptor loss that persists through six to seven population doublings. The data also indicate that recovery from receptor loss is a function of dose. The mechanism of this prolonged loss of receptors is yet to be defined.

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