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Distinct Regulation of β_1- and β_2-Adrenergic Receptors in Chinese Hamster Fibroblasts

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SUMMARY

The agonist-induced reduction of β-adrenergic receptor (βAR) cell surface density is a well documented phenomenon. The mechanisms responsible for this regulation have been well characterized for the β_2AR. They include a rapid sequestration of the receptor away from the cell surface in a vesicular compartment and a longer term down-regulation of the total β_2AR number. In contrast, very little is known about the cell surface regulation of the β_1AR. In the present study, we have compared the agonist-mediated regulation of β_1- and β_2AR in Chinese hamster fibroblasts transfected with the cDNA encoding either βAR subtype. Cells expressing similar numbers of the two βAR subtypes were selected for the study. The expressed receptors exhibit typical β_1- and β_2AR selectivity for agonists and antagonists, as assessed by radioligand binding. Both receptors were found to be positively coupled to the adenylyl cyclase stimulatory pathway, but marked differences in the receptor regulation profiles were observed. Treatment of the cells expressing the β_2AR with the agonist isoproterenol leads to a rapid sequestration of >30% of the receptors away from the cell surface into a light vesicular fraction, where they are inaccessible to the hydrophilic ligand CGP-12177. In contrast, virtually no agonist-induced sequestration is observed in the cells expressing the β_1AR. Longer exposure of the cells to isoproterenol leads to a time-dependent reduction in the total number of βARs in both β_1- and β_2AR-expressing cell lines. However, this down-regulation is significantly slower in the cells expressing the β_1AR. In fact, no appreciable down-regulation of the β_1ARs is detected in the first 4 hr of agonist treatment, compared with a down-regulation of >50% of the β_2ARs for the same period. After a 24-hr treatment with isoproterenol, <20% of the original number of β_2ARs remain, whereas 60% of the β_1ARs are still present after the same treatment. These results, therefore, suggest that, when expressed in an identical cell line, β_1AR and β_2AR follow distinct patterns of regulation. In fact, both agonist-induced sequestration and down-regulation are considerably blunted for the β_1AR, compared with the β_2AR.

Sustained hormonal stimulation has been shown to lead to a decreased responsiveness in many tissues and cell lines. This phenomenon, known as agonist-induced desensitization, has been shown, in several instances, to involve a loss in cell surface receptors (1). The processes responsible for such an adaptive mechanism have been best studied for the adenylyl cyclase-coupled β_2AR (for a review, see Ref. 2). At least two distinct processes are believed to contribute to the reduction of β_2AR number accompanying the agonist-mediated desensitization of the β_2AR/adenylyl cyclase system; a rapid (minutes) sequestration of the receptor away from the plasma membrane into a light vesicular fraction is followed by a longer term (hours) down-regulation of the total receptor number. The sequestration, therefore, leads to a reduction in the cell surface β_2AR number with no change in the total cellular receptor contingent, whereas the down-regulation is characterized by a net reduction of the total receptor number. Recent studies have proposed that the down-regulation process could result in part from an agonist-mediated reduction in the steady state level of the β_2AR mRNA, which would lead to a reduced rate of receptor synthesis (3–5). Such a decrease in receptor synthesis alone, however, cannot account for all of the down-regulation, and an active degradation of the β_2AR is believed to play an important role in this regulatory process (6–8).

In contrast to the well documented desensitization of the β_2AR, very little is known about the regulation of the β_1AR subtype responsiveness. Indeed, much of the progress in understanding the processes of β_2AR desensitization has come from studies conducted with established cell lines expressing the β_2AR subtype. The lack of such a cell line expressing a pure population of β_1AR has considerably hampered our progress.

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ABBREVIATIONS: βAR, β-adrenergic receptor; [^{125}I]CYP, [^{125}I]iodocyanopindolol; CHW, Chinese hamster fibroblast; DMEM, Dulbecco's minimum Eagle's medium; PBS, phosphate-buffered saline; Gpp(NH)p, guanosine 5′-(β,γ-imido)triphosphate.

However, work performed with cell lines and tissues expressing a mixed population of β_1- and β_2ARs has led us to suggest that these two receptor subtypes may have distinct desensitization patterns. In C_6 glioma cells, which express both subtypes of receptor, the atypical partial agonists celiprolol and pindolol have been shown to down-regulate the number of β_2ARs but not of β_1ARs (9). In the same cell line, it has also been demonstrated that, during the recovery from agonist-induced down-regulation, the half-life of the β_1AR is much longer than that of the β_2AR (10). More recently, *in vivo* infusion of isoproterenol in rat has been shown to induce a significantly higher down-regulation of the β_2AR than of the β_1AR in cardiac tissue (11).

It should be emphasized that the two βAR subtypes are the products of two different genes and that, in humans, for example, overall homologies at the amino acid level are only 54% (12). Such a structural difference could harbor the molecular determinants for distinct regulation patterns. The present study was, therefore, aimed at determining whether the cell surface densities of β_1- and β_2AR are under the control of distinct regulatory processes. To address this question, cell lines expressing either a pure β_1AR or β_2AR population were generated by transfecting human cDNAs encoding the β_1- or the β_2AR in CHW cells. The agonist-induced βAR sequestration and down-regulation patterns observed in cells bearing the β_1 or the β_2 subtypes were then compared. The results show that the human β_1- and β_2AR expressed in the same foster cell line have distinct patterns of cell surface regulation upon agonist stimulation.

Experimental Procedures

Materials. [^{125}I]CYP, [^{125}I]-pindolol, [α-^{32}P]ATP, and [^{3}H]cAMP were obtained from New England Nuclear. Isoproterenol, norepinephrine, epinephrine, ATP, GTP, cAMP, phosphoenolpyruvate, and myokinase were purchased from Sigma. Pyruvate kinase and isobutylmethylxanthine were from Calbiochem. G-418, DMEM, fetal calf serum, penicillin, streptomycin, amphotericin B, and trypsin were purchased from GIBCO. Alprenolol and CGP-12177 were generous gifts of Hassle Pharmaceutical Co. (Mölndal, Sweden) and Ciba Geigy, respectively.

DNA constructions, cell transfection, and culture. The entire human β_1AR cDNA, including eight bases of 5′ untranslated region (12), and the human β_2AR cDNA clone pTF (13) were inserted into the *Bgl*II site of the eukaryotic expression vector pKSV-10 (Pharmacia LKB Biotechnology, Inc.). These constructs were cotransfected with the neomycin resistance plasmid pSV2-Neo (Pharmacia LKB Biotechnology, Inc.) into CHW 1102 cells, by the calcium phosphate precipitation procedure (14). Neomycin-resistant cells were selected by culturing in DMEM plus 10% fetal calf serum containing 150 μg/ml G418. Clones were then screened for βAR expression by radioligand binding assays, using [^{125}I]CYP as the ligand. Cell lines expressing similar number of receptors (~450 fmol/mg of membrane protein) were selected for the study, and no significant difference in the level of expression between the two receptor subtypes was observed during the study. The transfected CHW cells were grown as monolayers in 75-cm^2 flasks containing DMEM supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphoterecin B, and 1 mM glutamine, in an atmosphere of 95% air and 5% CO_2, at 37°.

Whole-cell radioligand binding assays. Nearly confluent cells were incubated at 37° for the indicated periods of time with DMEM supplemented as described above and containing isoproterenol (1 μM). Control cells were incubated with the supplemented medium alone. After incubation, the cells were washed twice with PBS, detached with trypsin (0.25%), washed three times with supplemented DMEM, and resuspended in PBS at a concentration of 1.0 mg of protein/ml; 0.05 ml of cell suspension was used with 0.2 μCi of [^{125}I]-pindolol. Binding reactions were performed in DMEM at 13° for 3.5 hr or at 25° for 1.5 hr, in a final volume of 0.5 ml, and were terminated by rapid filtration over GF/C glass fiber filters (Whatman). Total cellular βAR was defined as the amount of radioligand binding inhibited by 0.3 μM (−)-propranolol, whereas cell surface receptor number was defined as the amount of [^{125}I]-pindolol binding inhibited by 0.1 μM CGP-12177 at 13°.

Membrane preparations and radioligand binding assays. Cells were washed three times with 5 ml of PBS, at room temperature, and were mechanically detached in 10 ml of ice-cold buffer containing 5 mM Tris·HCl (pH 7.4), 2 mM EDTA, 5 μg/ml leupeptin, 5 μg/ml soybean trypsin inhibitor, and 10 μg/ml benzamidine. Cells were lysed with a Polytron homogenizer (Janke & Kunkel) (one 5-sec burst at maximum speed). The lysate was centrifuged at 45,000 × *g* for 20 min at 4° and washed twice in the same buffer. The pelleted membranes were resuspended to a concentration of ~1.5 mg/ml in a buffer containing 75 mM Tris·HCl (pH 7.4), 12.5 mM $MgCl_2$, and 2 mM EDTA. These membrane preparations were used for adenylyl cyclase activity determination and radioligand binding assays as described below.

For subcellar distribution experiments, after incubation for the indicated periods of time with isoproterenol the cells were incubated with 0.25 mg/ml concanavalin A in PBS for 20 min on ice. The cells were then homogenized as described above, the lysate was centrifuged at 200 × *g* for 10 min at 4°, and the supernatant was layered on top of a 35% sucrose cushion and centrifuged at 150,000 × *g* for 90 min. As previously reported (15), the 0–35% interface contains the light membrane vesicular fraction, whereas the plasma membrane fraction sediments at the bottom of the sucrose cushion. Each membrane fraction was collected, diluted in 5 mM Tris·HCl (pH 7.4), 2 mM EDTA, and centrifuged at 200,000 × *g* for 60 min. The pelleted membranes were resuspended in 75 mM Tris·HCl (pH 7.4), 12.5 mM $MgCl_2$, 2 mM EDTA, and used immediately for radioligand binding assays.

Radioligand binding assays were conducted using 0.01 ml of membrane suspension (~15 μg of protein) in a total volume of 0.5 ml. For saturation experiments, duplicate assay tubes contained 2–400 pM [^{125}I]CYP, in the presence or absence of 10 μM alprenolol to define nonspecific binding. For competition experiments, duplicate assay tubes contained ~50 pM [^{125}I]CYP and 0–100 μM displacing agents (isoproterenol, norepinephrine, epinephrine, alprenolol, betaxolol, and ICI-118551). For competition assays with agonists, 100 μM Gpp(NH)p was added to the incubation mixture. The binding reactions were terminated by rapid filtration over GF/C glass fiber filters (Whatman). Data from competition and saturation experiments were analyzed by nonlinear least-squares regression, using the computer program LIGAND (16). For routine determination of receptor number, triplicate assay tubes contained a saturating concentration of [^{125}I]CYP (400 pM), in the presence or absence of 10 μM alprenolol.

Adenylyl cyclase assays. Adenylyl cyclase activities were measured by the method of Salomon *et al.* (17). Assay mixtures contained 0.02 ml of membrane suspension, 0.012 mM ATP, 0.1 mM cAMP, 0.053 mM GTP, 2.7 mM phosphoenolpyruvate, 0.2 unit of pyruvate kinase, 1 unit of myokinase, and 0.13 μCi of [α-^{32}P]ATP, in a final volume of 0.05 ml. Enzyme activity was determined in the absence of activator (basal activity) or in the presence of 0–100 μM isoproterenol or 10 μM forskolin. Reactions were initiated by the addition of the membranes, and the assay mixture was incubated for 30 min at 37°. Reactions were terminated by the addition of 1 ml of an ice-cold solution containing 0.4 mM ATP, 0.3 mM cAMP, and [^{3}H]cAMP (25,000 cpm). The [^{32}P]cAMP was isolated by sequential chromatography on a Dowex cation exchange resin and aluminum oxide.

Results

Expression of β_1- and β_2AR in CHW cells. CHW cells with no detectable βAR-stimulated adenylyl cyclase activity

were transfected with cDNAs encoding the human β_1- or β_2AR. Cellular clones were selected by virtue of their co-transfection with the G-418 resistance marker, and βAR expression was assessed in the resistant clones by [^{125}]CYP binding assays. Cell lines expressing similar levels of receptors (300–600 fmol/mg of membrane protein) were chosen for the study.

A pharmacological characterization of the [^{125}I]CYP binding sites expressed in the transfected CHW cells was performed. K_d values of 49 pM and 61 pM for [^{125}I]CYP were found in cells transfected with the β_2- and the β_1AR cDNAs, respectively, in good agreement with the K_d values reported for these receptors in other transfected cell lines (12, 18, 19). It is noteworthy that these K_d values are slightly higher than those reported in many cell lines naturally expressing those receptors. As seen in Fig. 1, competition experiments confirmed the pharmacological nature of the [^{125}I]CYP binding sites expressed in the transfected cells. The order of potencies of adrenergic agonists to inhibit [^{125}I]CYP binding in cells transfected with the β_2AR cDNA is (−)-isoproterenol > (−)-epinephrine > (−)-norepinephrine, whereas for antagonists the order is alprenolol > ICI-118551 > betaxolol. This pharmacological profile is consistent with the expression of a βAR of the β_2 subtype (20). In cells transfected with the β_1AR cDNA, the order of potencies of βAR agonists and antagonists is as follows: (−)-isoproterenol > (−)-norepinephrine > (−)-epinephrine and alprenolol > betaxolol > ICI-118551, consistent with a β_1AR pharmacology (20). When the competition assays with isoproterenol were conducted for both receptors in the absence of Gpp(NH)p, the displacement curves could be best fitted to a two-site model, using the nonlinear least-squares regression computer program LIGAND (data not shown), suggesting a functional coupling of the two receptor subtypes to a GTP-binding protein.

Adenylyl cyclase stimulation. To determine whether the two βAR subtypes could productively interact with the adenylyl cyclase stimulatory system in CHW cells, the ability of receptors expression to confer an isoproterenol-sensitive adenylyl cyclase activity to these cells was tested. As shown in Fig. 2, isoproterenol can stimulate the adenylyl cyclase activity in a dose-dependent fashion in membranes derived from cells transfected with either the β_1- or the β_2AR cDNA. No such stimulation could be detected in untransfected cells (data not shown). In cell lines expressing similar number of receptors (551 fmol/mg of membrane protein for β_1AR versus 690 fmol/mg of membrane protein for β_2AR), the abilities of the two receptors to stimulate the adenylyl cyclase activity are indistinguishable. Indeed, the maximal isoproterenol-stimulated level of the adenylyl cyclase and the EC_{50} of the enzyme stimulation by the nonselective βAR agonist are identical in the cells expressing the β_1- and the β_2AR. Forskolin also equally stimulates the adenylyl cyclase activity in both cell lines (data not shown).

Agonist-induced sequestration. Incubation of the β_2AR-expressing cells with isoproterenol leads to a rapid and time-dependent decrease in the number of [^{125}I]-pindolol binding sites displaced by the hydrophilic ligand CGP-12177 (0.1 μM), with no change in the number of [^{125}I]-pindolol binding sites displaced by the lipophilic antagonist propranolol (0.1 μM).

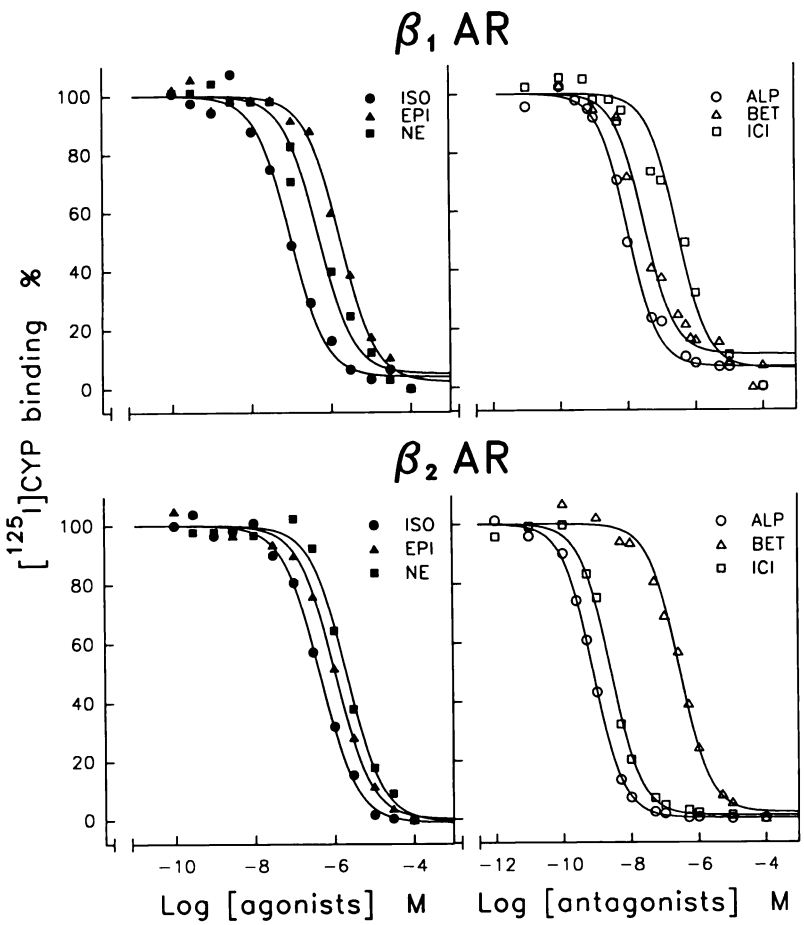


Fig. 1. Competition of [^{125}I]CYP binding with βAR agonists and antagonists. Membranes derived from CHW cells transfected with the human β_1AR cDNA (*β_1AR*) or the human β_2AR cDNA (*β_2AR*) were prepared and assayed for [^{125}I]CYP binding, as described in Experimental Procedures. The binding of [^{125}I]CYP was assessed in the presence of varying concentrations of the agonists isoproterenol (*ISO*), epinephrine (*EPI*), and norepinephrine (*NE*) and of the antagonists alprenolol (*ALP*), betaxolol (*BET*), and ICI-118551 (*ICI*). The data were analyzed using the nonlinear least-squares regression computer program LIGAND (16). The results shown are representative of two distinct experiments conducted in triplicate. Calculated K_i values were, for the β_1AR, isoproterenol, 50 nM; epinephrine, 900 nM; norepinephrine, 250 nM; alprenolol, 5.0 nM; betaxol, 15 nM; and ICI-118551, 150 nM; and, for the β_2AR, isoproterenol, 140 nM; epinephrine, 370 nM; norepinephrine, 740 nM; alprenolol, 0.27 nM; betaxolol, 100 nM; and ICI-118551, 0.93 nM.

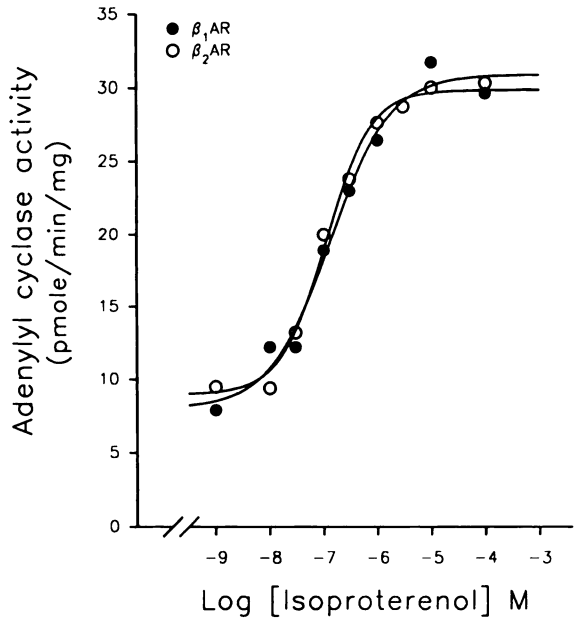


Fig. 2. Dose-response curves for the isoproterenol stimulation of the adenylyl cyclase of the β_1- and β_2AR-expressing CHW cells. The adenylyl cyclase activity was measured in membrane preparations as described in Experimental Procedures and is expressed as pmol of cAMP produced/min × mg of protein. The data were analyzed using nonlinear least-squares regressions, and the EC_{50} values calculated were 0.9×10^{-7} M and 1.2×10^{-7} M for the β_1- and β_2AR, respectively. The data shown are representative of two separate experiments conducted in duplicate.

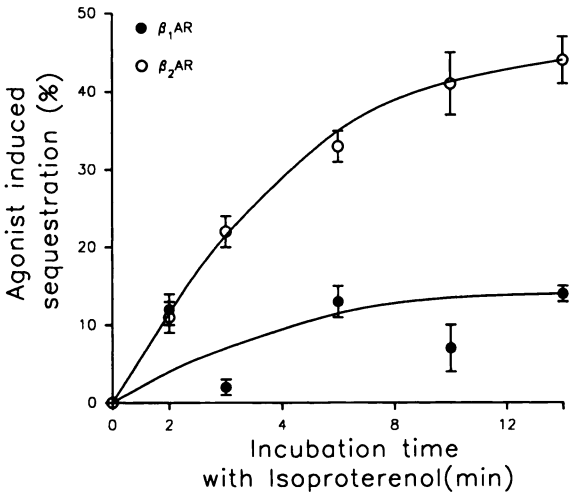
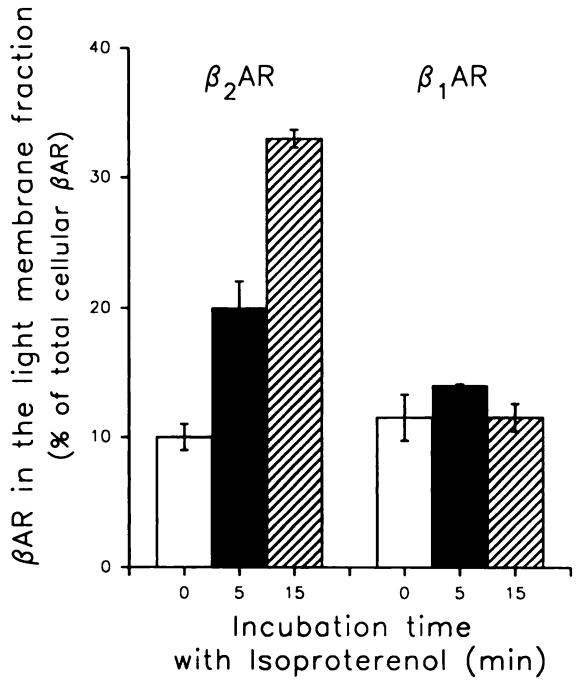


Fig. 3. Isoproterenol-induced sequestration of the βARs in CHW cells expressing the β_1 and β_2 subtypes. Cells were incubated with 1 μM isoproterenol at 37° for the indicated times. Sequestration is defined as the difference between the total number of specific [^{125}I]-pindolol binding sites and the cell surface sites accessible to CGP-12177, as described in Experimental Procedures. The isoproterenol-induced sequestration is expressed as percentage of total βAR receptor number. Data are mean ± standard error of three separate experiments conducted in triplicate.

This change in the accessibility of the [^{125}I]-pindolol binding sites to a hydrophilic ligand is interpreted as a sequestration of the β_2AR away from the cell surface into a cytoplasmic compartment (21). As shown in Fig. 3, this agonist-induced sequestration of the receptor can be expressed as a percentage of the total receptor number detected in the cells and, after 16 min of exposure to isoproterenol, >40% of the β_2ARs are sequestered away from the cell surface. In contrast, exposure of the cells expressing the β_1AR to isoproterenol for the same period of time leads to very little change in the number of sites accessible to CGP-12177. In fact, the 16-min treatment with isoproterenol induces a sequestration of <10% of the β_1ARs (Fig. 3). This difference is not due to a selective affinity of CGP-12177 for the β_2AR versus the β_1AR, because the K_i of CGP-12177 towards [^{125}I]-pindolol binding is the same in the β_1- and β_2AR-expressing cells (data not shown).

To verify that this difference in the agonist-induced change in accessibility to CGP-12177 truly corresponds to a difference in the sequestration profile of the two receptors, the physical translocation of the receptors from the plasma membrane into the sequestered vesicular compartment was assessed directly. The numbers of receptors present in the plasma membrane and in a light membrane fraction, believed to contain the sequestered receptors (21, 22), were determined, as described in Experimental Procedures, before and after a treatment of 5 or 15 min with isoproterenol. As shown in Fig. 4, in cells expressing the β_2AR the agonist treatment induces a gradual increase in the proportion of the total cellular receptors present in the light membrane fraction, with no change in the total number of receptors. The amplitude of this time-dependent translocation is consistent with the extent of loss in CGP-12177-accessible β_2AR described above. In sharp contrast, when cells expressing the β_1AR are exposed to isoproterenol for 5 or 15 min, no change in the proportion of β_1AR present in the light membrane subcellular fraction is observed (Fig. 4). This absence of translocation is consistent with the very marginal changes in the accessibility of the β_1AR to CGP-12177 observed after agonist treatment.



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membrane into a light vesicular fraction. Cells were incubated with 1 μM isoproterenol at 37° for the indicated times. Receptor numbers were measured in the plasma membrane fraction and in a light membrane fraction, after differential centrifugation, in a [^{125}I]CYP binding assay, as described in Experimental Procedures. The data are expressed as a percentage of the total receptors present in the light membrane fraction and represent the mean ± standard error of three separate experiments.

Agonist-induced down-regulation. Treatment of CHW cells expressing the β_2AR with isoproterenol for 2–24 hr leads to a time-dependent reduction of the total number of β_2ARs. Indeed, the [^{125}I]-pindolol binding sites displaced by the lipophilic antagonist propranolol in whole-cell binding assays decrease sharply after long term agonist treatment in cells transfected with the β_2AR cDNA (Fig. 5). In the first 4 hr of treatment, the number of β_2ARs decreases to 42% of the control value. This down-regulation then slowly progresses with time and, after a 24-hr incubation with isoproterenol, >80% of the β_2AR binding sites have disappeared from the cells. When the cells expressing the β_1AR are exposed to the same treatment with isoproterenol, a strikingly different pattern of down-regulation is observed (Fig. 5). In fact, the decrease in [^{125}I]-pindolol specific binding sites is significantly slower in these cells, and no consistent down-regulation is observed in the first 4 hr of isoproterenol treatment. A slowly evolving down-regulation then takes place, and the number of β_1ARs reaches 52% of the control value after 24 hr of agonist treatment.

To test the possibility that residual isoproterenol could interfere with the binding of the radioligand, thus affecting the receptor number determination, complete [^{125}I]CYP saturation isotherms were measured in cells treated for 4 hr with either isoproterenol or the vehicle alone. The apparent K_d for [^{125}I] CYP was found not to be affected by the isoproterenol treatment (β_1AR: control, 79 pM; isoproterenol, 80 pM; β_2AR: control, 42 pM; isoproterenol, 48 pM), suggesting that no residual isoproterenol is present during the binding assay.

Discussion

The results presented here demonstrate that, when expressed in the same foster cell line (CHW), the human β_1- and β_2AR have distinct regulation patterns. In fact, although the two receptor subtypes have identical capacities to stimulate the adenylyl cyclase signaling pathway in these cells, the agonist-mediated regulation of receptor number and subcellular distribution were found to be very different in the β_1- and β_2AR-expressing cells. First, the rapid and important agonist-promoted sequestration of the receptor away from the cell surface observed in the β_2AR-expressing cells was not found in the cells expressing the β_1AR. Second, the longer term down-regulation of the total β_1AR number was found to be much slower and of lesser amplitude than that of the β_2AR.

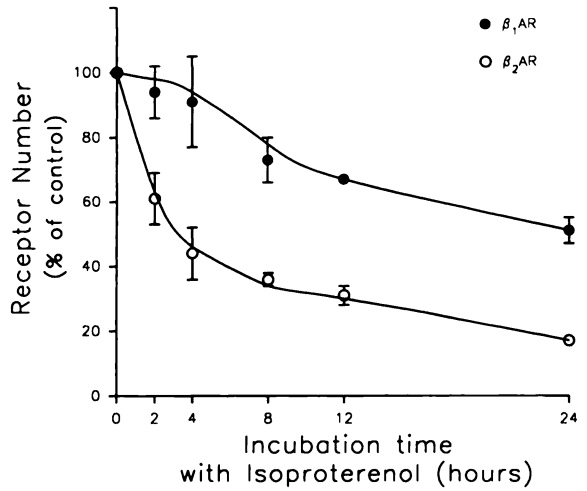

Fig. 5. Isoproterenol-induced down-regulation of βARs in CHW cells expressing the β_1 and β_2 subtypes. Cells were incubated with 1 μM isoproterenol for 0–24 hr at 37°, and whole-cell βAR number was determined by radioligand binding, using ^{125}I-pindolol, as described in Experimental Procedures. Data are mean ± standard error of three separate experiments conducted in triplicate and are expressed as percentage of the number of receptors in control cells.

The virtual absence of agonist-induced sequestration of the β_1AR in CHW cells was confirmed by two different approaches. Indeed, short term (0–15 min) treatment with isoproterenol did not induce any translocation of the β_1AR from the plasma membrane to the light vesicular fraction when the two fractions were separated by differential centrifugation. Similarly, only a marginal agonist-promoted change in the accessibility of the β_1AR to a nonpermeable ligand (CGP-12177) could be detected in whole-cell binding assays during a 16-min exposure to isoproterenol. This contrasts sharply with the important sequestration of the β_2AR observed, using either technique, in the β_2AR-expressing CHW cells.

The major difference in the patterns of agonist-induced down-regulation between the β_1- and the β_2AR resides in the absence, in the β_1AR down-regulation curve, of a rather rapid component that leads to the loss of >50% of the β_2ARs within the first 4 hr of agonist treatment. It could be hypothesized that this altered down-regulation pattern results from the virtual absence of agonist-induced sequestration observed for the β_1AR. Indeed, it has been proposed that these two processes could be sequential (23, 24). In such a model, the sequestered receptors are internalized in a compartment similar to the endosomes and are ultimately degraded in the lysosomes, much like the transferrin receptor and many growth factor receptors. It follows that a blunted agonist-induced sequestration, such as the one observed for the β_1AR, would lead to a reduced degradation and, hence, down-regulation of this βAR subtype, compared with the β_2AR. However, several lines of evidence suggest that agonist-induced sequestration and down-regulation could be, at least in part, nonsequential events. These include the observation that permeable cAMP analogues (4) and partial agonists (25) do not elicit any β_2AR sequestration but do lead to a partial down-regulation. More recently, a site-directed mutation of the β_2AR was shown to block the agonist-promoted sequestration of the receptor completely, without affecting its down-regulation (26). The link between the absence of a consistent agonist-induced sequestration and the slow down-regulation observed in the β_1AR-expressing cells, therefore, remains to be evaluated.

Several studies have suggested the existence of distinct regulation for the β_1- and β_2AR under different conditions. Prolonged *in vivo* infusion of isoproterenol in rats has been shown to induce a down-regulation of >70% of the heart β_2AR, with a concomitant reduction of <40% of the β_1AR (11). In C_6 glioma cells, the half-life of the β_1AR was found to be much longer than that of the β_2AR during recovery from down-regulation (10), consistent with the existence of distinct regulation for the two receptor subtypes. Similarly, the partial agonist celiprolol was found to induce a significant down-regulation of β_2ARs, without affecting the number of β_1ARs, in C_6 glioma cells (9). Until recently, however, the absence of established cell lines expressing only the β_1AR subtype has rendered the study of β_1AR regulation difficult. In a recent report, Fishman *et al.* (27) characterized a human neuroepithelioma cell line that appears to express a pure population of

β_1AR. The long term down-regulation of the β_1AR in that cell line appeared to be of lesser amplitude than the down-regulation of the β_2AR observed in cell lines such as S49 mouse lymphoma or DDT$_1$MF-2 smooth muscle cells. It is, however, difficult to determine whether the difference reported in this study is intrinsic to the receptor subtype or merely reflects a property of the neuroepithelioma cell line. In the present study, the fact that the two receptor subtypes are individually expressed in the same foster cell line allows us to conclude that the differences observed in their agonist-induced sequestration and down-regulation patterns represent intrinsic properties of the β_1ARs.

It has recently been shown that, when expressed in murine L cells, the turkey βAR is not susceptible to agonist-promoted sequestration or down-regulation (28). Interestingly, the turkey βAR shares 69% of overall identity with the mamalian β_1AR and displays selectivity among the βAR ligands that is closer to that of the β_1AR than to that of the β_2AR. A recombinant mutation that introduced a truncation in the carboxyl tail of the turkey βAR, along with the insertion of 12 new carboxyl-terminal amino acids, produced agonist-promoted sequestration and down-regulation of the receptor. This suggests the importance of the carboxyl-terminal domain of the βAR in determining these processes.

We recently reported that the mutation of two tyrosine residues in the carboxyl tail of the β_2AR (tyrosine-350 and -354) considerably slowed and decreased the amplitude of its agonist-mediated down-regulation (29). When the sequences of the β_1AR and β_2AR cytoplasmic tails are compared, it is noteworthy that, whereas 73% identity is found in the first 15 amino acids after the proposed seventh transmembrane domain of the receptors, this number falls to 0% in the next 15 amino acids. This second region harbors the two tyrosines that were proposed to play an important role in the agonist-induced down-regulation of the β_2AR. In fact, no tyrosine residue is present in the entire cytoplasmic tail of the β_1AR. Interestingly, the down-regulation pattern of the β_1AR is virtually identical to the one observed for the β_2AR lacking tyrosine-350 and -354 ([Ala^{350},Ala^{354}]β_2AR), reported by Valiquette *et al.* (29).

Regulation of the β_2AR mRNA level has recently been suggested to contribute to the long term agonist-induced down-regulation of the receptor (3–5). On the other hand, distinct regulation of the β_1- and β_2AR mRNAs during 3T3 adipocyte differentiation and upon dexamethasone treatment has been reported (30, 31). Dexamethasone treatment of 3T3-F442A adipocytes represses the expression of the β_1AR mRNA while strongly stimulating the expression of the β_2AR mRNA. Differential regulation of the β_1- and β_2AR mRNA levels could, therefore, also contribute to the elaboration of different agonist-induced down-regulation patterns.

Distinct regulation of the β_1- and β_2AR may have important physiological and even therapeutic consequences. For instance, alteration of the relative β_1- and β_2AR contribution to the inotropic response to catecholamines has been reported in human and experimental heart failure (32, 33). Thus, the occurrence of selective regulation of the βAR subtypes could play important physiological roles.

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