A Truncation Mutation in the Avian β-Adrenergic Receptor Causes Agonist-induced Internalization and GTP-sensitive Agonist Binding Characteristic of Mammalian Receptors*

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Recombinant turkey erythrocyte β-adrenergic receptors expressed in murine L cells exhibited characteristic avian subtype selectivity for agonists and antagonists. In 10 of the 11 clones studied, no agonist-induced internalization of receptor was observed, although agonist-induced uncoupling of receptor and adenylyl cyclase occurred rapidly. GTP caused little or no decrease in affinity for β-adrenergic agonists. Such behavior is commonly observed in avian erythrocytes. In contrast, one clone was susceptible to agonist-induced receptor internalization and down-regulation even though it exhibited characteristic avian β-adrenergic ligand-binding properties. The affinity of this variant receptor for agonists was also notably reduced by GTP. Electrophoresis of affinity-labeled receptor from this clone indicated an apparent size of about 33 kDa, about 12 kDa less than that of the native or recombinant turkey β-adrenergic receptor. Genomic DNA from this cell line that encodes the receptor was cloned and partially sequenced. The coding region of the original receptor cDNA was interrupted after codon 412 (out of 483) and was followed by 36 base pairs of novel sequence prior to the first in-frame stop codon. These results suggest that the lack of both hormone-induced internalization and down-regulation even though it exhibited characteristic avian β-adrenergic ligand-binding properties. The affinity of this variant receptor for agonists was also notably reduced by GTP. While no significant differences between mammalian β- and β2-adrenergic receptors have been observed in the reactions subserving desensitization, the avian receptor desensitizes by a distinctly different mechanism (2). Three sets of reactions contribute to agonist-induced desensitization of mammalian receptors (see Refs. 2-4 for reviews). Receptors are phosphorylated by both cyclic AMP-dependent protein kinase and β-adrenergic receptor kinase. They are sequestered in a process that may reflect endocytosis. They are also downregulated, observed as a nonreversible loss of binding sites for receptor-specific lipophilic ligands such as [125I]ICYP. 1 In turkey erythrocytes, a cyclic AMP-dependent phosphorylation of the receptor is thought to result in desensitization, but without consequent endocytosis or degradation (7, 8).

A second difference between avian and mammalian receptors is the effect of guanine nucleotides on the affinity with which agonists bind to the receptor. In mammalian cells, a Gα-receptor complex displays a higher affinity for agonists than does the isolated receptor, causing the apparent coexistence of high and low affinity sites for agonist binding. Guanine nucleotides dissociate the receptor-Gα complex and shift agonist binding exclusively to the low affinity state (see Refs. 9 and 10 for reviews). In contrast, the affinity of avian receptors for agonists is less obviously biphasic and effects of guanine nucleotides on agonist binding are difficult to detect except under special conditions (11-13).

Such differences in cellular behavior between avian and mammalian receptors could reflect either a distinctive structure of the avian receptor or a difference in the capabilities of avian and mammalian cells to regulate, modify, or process the receptor. To study this problem, we introduced cDNA that encodes the β-adrenergic receptor into murine L cells, regulated (2-4). Although the actions of β-adrenergic receptors are essentially the same in different tissues and species, differences in agonist- and antagonist-binding properties have allowed their further categorization into at least four subtypes, β1, β2, and β3 (5) in mammalian cells, and β1 in turkey erythrocytes, a popular model system for the study of β-adrenergic action. The mammalian subtypes display distinctively different selectivities among β-adrenergic ligands and responses to partial agonists. The β-adrenergic receptor of turkey reticulocytes and erythrocytes displays further subtle differences from the mammalian β1 receptor that can be observed clearly only by the use of certain selective antagonists (6).

Catecholamines act through β adrenergic receptors to promote the activation of the G protein Gα, which in turn stimulates the activity of adenylyl cyclase and several ion channels (see Ref. 1 for overview). A subsequent effect of agonist binding is the induction of receptor desensitization and down-regulation (2-4). Although the actions of β-adrenergic receptors are essentially the same in different tissues and species, differences in agonist- and antagonist-binding properties have allowed their further categorization into at least four subtypes, β1, β2, and β3 (5) in mammalian cells, and β1 in turkey erythrocytes, a popular model system for the study of β-adrenergic action. The mammalian subtypes display distinctively different selectivities among β-adrenergic ligands and responses to partial agonists. The β-adrenergic receptor of turkey reticulocytes and erythrocytes displays further subtle differences from the mammalian β1 receptor that can be observed clearly only by the use of certain selective antagonists (6).

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Such differences in cellular behavior between avian and mammalian receptors could reflect either a distinctive structure of the avian receptor or a difference in the capabilities of avian and mammalian cells to regulate, modify, or process the receptor. To study this problem, we introduced cDNA that encodes the β-adrenergic receptor into murine L cells,
which lack endogenous \( \beta \)-adrenergic receptors (14), and characterized the properties of the \( \beta \)-adrenergic receptor when it is expressed in a mammalian cell. A similar analysis of the properties of hamster \( \beta \)-adrenergic receptors expressed in mouse L cells also has been carried out.\(^2\) The comparison indicates that the desensitization properties characteristic of \( \beta \)-adrenergic receptors are a reflection of a receptor's structure and are not properties of the host cell.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dulbecco’s modified Eagle’s medium, fetal calf serum, and G418 were obtained from Gibco. \([\alpha-\text{\textsuperscript{32P}}]\)ATP was purchased from ICN Biochemicals. \((-\text{Isoproterenol, \(-\text{epinephrine, and \(-\text{norepinephrine were purchased from Sigma. The following drugs were gifts: IC1 118551 and IC1 89406 from IC1, Macclesfield, Great Britain; \((-\text{cyano})\)pinodolol and \((-\text{pinodolol from Dr. G. Engel, Sandoz, Basel, Switzerland; CGP12177 from Dr. M. Staeheli, Ciba Geigy AG, Basel, Switzerland. Cyanopindolol was iodinated to a specific radioactivity of 50 Bq/\text{mol by a modification of the method of Borovsky and Brocker (15). [\text{\textsuperscript{125I}}]Iodocyanopindololdiazetine was obtained from Amersham Corp.**

**Cell Culture**—Murine L cells (from Dr. N. Birnberg, Yale University) and 1321N1 human astrocytoma cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum under 95% air and 5% CO\(_2\) at 37 °C. Cells were seeded at 4 × 10\(^5\) cells/cm\(^2\) and were allowed to grow for 4 days prior to each experiment.

**Transfection and Expression**—The plasmid pSV\text{BAR} was constructed by inserting the complete cDNA that encodes the turkey erythrocyte \( \beta \)-adrenergic receptor (16) into the Pharmacia pKSV-10 expression vector. Twenty-four hours prior to transfection, \( 5 \times 10^6 \) L cells were plated in a 100-mm dish. Cells were transfected with pSV\text{BAR} (20 \\text{ug/dish}) and the selection marker pRSV\text{neo} (1 \\text{ug/dish}) by the calcium phosphate precipitation method (17). G418-resistant cells were selected using 800 Kg/ml G418. Individual drug-resistant colonies were isolated after about 2 weeks and maintained in Dulbecco’s modified Eagle’s medium plus 10% fetal calf serum and 300 \mu g/ml G418.

**DNA Sequencing**—Manipulation of recombinant DNA followed standard procedures (18). Genomic DNA from the LSV22 cell line was purified and cut with EcoRI. Southern blots probed with \( \text{\textsuperscript{32P}} \)-labeled cDNA that encodes the avian \( \beta \)-adrenergic receptor (19) displayed a prominent band at about 3 kilobase pairs. DNA was extracted from this region of an agarose gel and used to construct a library in pUC18. The library was probed with randomly primed (19), full-length cDNA. A single positive colony was selected, subcloned, and used to prepare double-stranded DNA for sequencing using Sequenase (U. S. Biochemicals) according to the manufacturer’s instructions.

**Binding Assays**—\( \beta \)-Adrenergic ligand binding was measured using hypotonic lysates prepared in 1 mm Tris-Cl and 2 mm EDTA, pH 7.2. Binding assays were performed in a solution of 154 mm NaCl, 5 mm MgCl\(_2\), 20 mm Tris-Cl, pH 7.4, that contained 50 pM \([\text{\textsuperscript{32P}}]\)ICYP. Internalized receptors were assayed with 10 pm \([\text{\textsuperscript{125I}}]\)ICYP and a range of \( \text{\textsuperscript{32P}}\)ATP concentrations (see Fig. 1 for examples; Refs. 9 and 10 for review). The high affinity state of avian receptors is, at best, difficult to observe, and consequently, guanine nucleotides decrease affinity for agonists only slightly. As shown in Fig. 1, GTP had little if any effect on the affinity of endogenous \( \beta \)-adrenergic receptor expressed in the A8 clone of L cells and for the \( \beta \)-adrenergic receptor expressed in 1321N1 human astrocytoma cells. The agonist-binding properties of hamster \( \beta \)-adrenergic receptors expressed in the transfected cells displayed \( \beta \)-selectivity for agonists.

**Activation of Adenylyl Cyclase**—Adenylyl cyclase activity was determined in cell lysates by the method of Salomon et al. (23), which involves the separation of \( \text{\textsuperscript{32P}}\)AMP from \( \text{\textsuperscript{32P}}\)ATP on a sequential column of Dowex 50 and alamime.

**Internalization of Receptors**—The internalized form of the \( \beta \)-adrenergic receptor was detected by two different methods. 1) Surface density gradient centrifugation. Prior to lysis, cells were incubated with 0.25 mg/ml concanavalin A for 20 min at 4 °C (24). Lysates (6.0 ml) were layered on top of a gradient of 20 to 50% sucrose (w/v) in 20 mm Tris-Cl, pH 7.4. Centrifugation was for 60 min at 25,000 rpm in a Beckman SW28 rotor. Fractions (1.5 ml) were collected in an ISCO model 568 fractionator. To determine the number of receptors/fraction, samples were diluted 1:1 with 154 mm NaCl, 20 mm Tris-Cl, 5 mm MgCl\(_2\), pH 7.4, and incubated with \([\text{\textsuperscript{125I}}]\)ICYP. Internalized receptors migrate preferentially at the 10–30% interface and plasma membrane-associated receptors migrated at 40–45% sucrose. 2) Competition with CGP12177. Lysates from control or isoproterenol-treated cells were assayed with 10 pm \([\text{\textsuperscript{125I}}]\)ICYP and a range of concentrations of CGP12177. Receptors in vesicles exhibited a marked inaccessibility to CGP12177 as described previously (25, 26). The increase in radioligand binding in the presence of CGP12177 thus provided a quantitation of internalized \( \beta \)-adrenergic receptors (27).

**RESULTS**

After transfection with the cDNA that encodes the \( \beta \)-adrenergic receptor, about 10% of the G418-resistant L cell clones displayed \( \beta \)-adrenergic ligand-binding activity. The extent of expression varied between 1 and 300 fmol of ICYP-binding sites/mg of protein in lysates from 10 randomly selected positive clones. The experiments shown below compare several clones that express different numbers of receptors within this range.

**Recombinant \( \beta \)-Adrenergic Receptors Display Avian Selectivity for Ligands**—The selectivity of recombinant receptors for \( \beta \)-adrenergic ligands was examined for 7 of the 10 selected clones. Of the four clones examined in detail for agonist-binding properties, all displayed characteristic \( \beta \)-adrenergic selectivity for agonists. Isoproterenol bound with the highest affinity and epinephrine bound with slightly lower affinity than did norepinephrine (Table I). The recombinant receptors bound the \( \beta \)-specific antagonist ICI 118551 with relatively low affinity, but they also displayed relatively low affinity for the \( \beta \)-specific antagonist ICI 89406. Furthermore, the receptors expressed in seven of seven clones tested bound pinodolol with a \( K_d \) in the nanomolar range (Table I) rather than in the picomolar range, which would have been characteristic of both mammalian \( \beta_1 \) and \( \beta_2 \) receptors. Thus, the receptors expressed in the transfected cells displayed \( \beta \)-selectivity for both agonist and antagonist binding (6).

**Effects of GTP on Agonist Binding**—The affinity of mammalian \( \beta \)-adrenergic receptors for agonist, but not antagonist, ligands is typically increased as a result of interaction with G\(_i\) and only the low affinity state of the receptor is observed in the presence of guanine nucleotides (see Fig. 1 for examples; Refs. 9 and 10 for review). The high affinity state of avian receptors is, at best, difficult to observe, and consequently, guanine nucleotides decrease affinity for agonists only slightly. As shown in Fig. 1, GTP had little if any effect on the agonist-binding affinities of the avian receptors in clones LSV7, 11, 30, and 49, as is generally observed in turkey erythrocyte membranes (11, 12). In contrast, receptors in clone LSV22 displayed an approximately 10-fold lower \( K_d \) for isoproterenol and GTP increased the \( K_d \) to about that observed in the other clones. For comparison, similar regulation by GTP is shown in Fig. 1 for the hamster \( \beta_2 \)-adrenergic receptor expressed in the A8 clone of L cells and for the endogenous \( \beta_2 \)-adrenergic receptor in 1321N1 human astrocytoma cells. The agonist-binding behavior of the receptors in clone LSV22 was characteristic of mammalian receptors.

**Activation of Adenylyl Cyclase**—Adenylyl cyclase activity

\(^2\) C. Hertel, N. Birnberg, E. A. Murphy, and J. P. Perkins, manuscript in preparation.
An Endocytosis-competent Avian $\beta$-Adrenergic Receptor

TABLE I

Equilibrium binding constants ($K_d$) for agonists and antagonists

Receptor number and affinity for $[^{38}S]$ICYP were determined by Scatchard analysis of equilibrium binding experiments. The apparent dissociation constants for the nonradioactive ligands were determined by competition with 50 pM $[^{38}S]$ICYP. Data were analyzed as described previously (26). Total numbers of receptors (fmol/mg of protein) were: LSV,7, 48 ± 12; LSV,11, 17 ± 2; LSV,13, 2.4 ± 1.2; LSV,22, 69 ± 5; and LSV,49, 20 ± 4. Receptors in clones LSV,10 and LSV,30 also were examined in terms of affinity for binding of pindolol; the results were similar to those for the recombinant $\beta$-adrenergic receptors in the other clones (data not shown).

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>LSV,7</th>
<th>LSV,11</th>
<th>LSV,13</th>
<th>LSV,22</th>
<th>LSV,49</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[^{38}S]$ICYP (pM)</td>
<td>5.5 ± 0.1</td>
<td>3.3 ± 0.3</td>
<td>2.6 ± 1.4</td>
<td>3.5 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Pindolol (nM)</td>
<td>5.0 ± 2.2</td>
<td>4.0 ± 3.0</td>
<td>1.6 ± 0.4</td>
<td>16 ± 5</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>ICI 118551 (nM)</td>
<td>—</td>
<td>73 ± 24</td>
<td>53 ± 12</td>
<td>47 ± 30</td>
<td>99 ± 23</td>
</tr>
<tr>
<td>ICI 89406 (nM)</td>
<td>—</td>
<td>86 ± 37</td>
<td>111 ± 11</td>
<td>77 ± 51</td>
<td>139 ± 11</td>
</tr>
</tbody>
</table>

Agonists

<table>
<thead>
<tr>
<th>Agonist</th>
<th>LSV,7</th>
<th>LSV,11</th>
<th>LSV,13</th>
<th>LSV,22</th>
<th>LSV,49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol (nM)</td>
<td>100 ± 2</td>
<td>80 ± 60</td>
<td>50 ± 10</td>
<td>33 ± 10</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>+ 0.1 mM GTP</td>
<td>120 ± 12</td>
<td>120 ± 70</td>
<td>70 ± 10</td>
<td>320 ± 180</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>Noradrenaline (nM)</td>
<td>—</td>
<td>870 ± 330</td>
<td>800 ± 400</td>
<td>310 ± 190</td>
<td>2100 ± 500</td>
</tr>
<tr>
<td>+ 0.1 mM GTP</td>
<td>—</td>
<td>1020 ± 1200</td>
<td>1400 ± 800</td>
<td>3200 ± 1900</td>
<td>2100 ± 500</td>
</tr>
<tr>
<td>Epinephrine (nM)</td>
<td>—</td>
<td>2300 ± 1200</td>
<td>400 ± 200</td>
<td>470 ± 270</td>
<td>2100 ± 1300</td>
</tr>
<tr>
<td>+ 0.1 mM GTP</td>
<td>—</td>
<td>2300 ± 1000</td>
<td>1400 ± 800</td>
<td>3200 ± 1900</td>
<td>2100 ± 500</td>
</tr>
</tbody>
</table>

FIG. 1. Effect of GTP on the binding of isoproterenol to $\beta$-adrenergic receptors. Isoproterenol binding was measured by competition with $[^{38}S]$ICYP as described under “Experimental Procedures,” in the presence (●) or absence (○) of 100 nM GTP. The data show the means of triplicate determinations of specific binding. One of two or three similar experiments is shown. The properties of 1321N1 human astrocytoma cells, which express $\beta$-adrenergic receptors, the A8 clone of L cells that express hamster $\beta$-adrenergic receptors, and four LSV clones (7, 11, 22, and 30) are compared.

was stimulated by $\beta$-adrenergic agonists in all L cells that expressed $\beta$-adrenergic receptors (Table II). The extent of stimulation varied from 2- to 12-fold. Clones LSV,22 and LSV,7, which expressed the highest numbers of receptors, also displayed the greatest stimulation by agonists, although there was no strict correlation between stimulation and the number of binding sites.

Desensitization—To investigate the mechanisms of ligand-induced desensitization of avian $\beta$-adrenergic receptors in L cells, we determined the effects of long term exposure to isoproterenol on responsiveness of adenyl cyclase to agonists, endocytosis of receptors, and down-regulation of receptors. All five clones tested (LSV,7, 11, 13, 22, and 49) exhibited a rapid ($t_{1/2} < 15$ min, not shown) and extensive (60-90%) ligand-induced reduction in the responsiveness of adenyl cyclase to isoproterenol (Fig 2).

Endocytosis and Down-regulation—When mammalian cells are exposed to $\beta$-adrenergic agonists, their receptors are ap-

FIG. 2. Adenylyl cyclase activity in LSV,7, LSV,11, LSV,22, and LSV,49. Adenylyl cyclase activity was determined in lysates from control cells (open bars) or cells treated with 1 $\mu$M isoproterenol for 20 min at 37°C (solid bars). Adenylyl cyclase activity in the presence of 1 $\mu$M isoproterenol is expressed as -fold stimulation over basal. Absolute values for basal activity in the different clones was: A, LSV,7, 0.8 ± 0.2, and LSV,22, 0.9 ± 0.01 pmol/min/mg; B, LSV,11, 6.1 ± 0.1 pmol/min/mg; LSV,13, 6.7 ± 0.6 pmol/min/mg; LSV,22, 2.4 ± 0.4 pmol/min/mg, and LSV,49, 4.0 ± 0.1 pmol/min/mg. Each experiment was done twice in triplicate. A and B indicate comparisons carried out as separate experiments 11 months apart.
An Endocytosis-competent Avian \( \beta \)-Adrenergic Receptor

are apparently endocytosed. They are found in less dense sucrose gradient fractions upon subcellular fractionation (24) and are inaccessible to hydrophilic ligands such as CGP12177 (25), suggesting that they have been relocated to intracellular vesicular structures. Subsequent to endocytosis, a net loss of receptors is observed, a process referred to as down-regulation (2, 28). Receptor endocytosis and down-regulation is not observed in turkey erythrocytes or reticulocytes, however (7, 8, 29).

As shown in Fig. 3, sucrose density gradient fractionation of recombinant avian \( \beta \)-adrenergic receptors from untreated L cells yielded a pattern typical of untreated mammalian \( \beta \)-adrenergic receptors. Fig. 3 shows examples for clones LSV,t7 and LSV,t22 in comparison with hamster \( \beta \)-adrenergic receptors, and similar patterns were observed for clones LSV,11, 13, and 49 (not shown). Receptors were found in two incompletely separated peaks, the larger usually at about 30-41% sucrose and the smaller at about 25-30% sucrose. As is the case with avian erythrocytes, exposure of clones LSV,7 (Fig. 3, bottom) or LSV,11, 13, or 49 (data not shown) did not alter the sedimentation pattern of receptors. Surprisingly, treatment of LSV,22 cells with isoproterenol did shift \( \beta \)-adrenergic receptors from high density to low density fractions (Fig. 3, middle). The hamster receptors behaved similarly (Fig. 3, bottom). Furthermore, the receptors found in the low density fractions after agonist treatment were largely inaccessible to CGP12177, suggesting an intravesicular location. The mammalian receptors in the low density fraction also were inaccessible to CGP12177.

The density gradient technique could not be applied readily to those clones that expressed a low number of \( \beta \)-adrenergic receptors. However, receptor endocytosis is also evident as a reduction in the potency with which CGP12177 competes with net ICYP binding, presumably because the binding sites of endocytosed receptors are oriented toward the lumen of the endocytic vesicles. Therefore, the accessibility of receptors in a total lysate was determined according to the ability of CGP12177 to compete with \([125I] ICYP for binding. As shown in Fig. 4, \( \beta \)-adrenergic receptor in clones LSV,7, 11, 30, and 49 did not become inaccessible to CGP12177 after the cells were treated with isoproterenol. Similar results were obtained for clones LSV,8, 10, and 51 (not shown). However, when LSV,22 cells were treated with isoproterenol, a fraction of

![Image](image-url)

**Fig. 3.** Sucrose density gradient centrifugation of \( \beta \)-adrenergic receptors in cell lysates. Untreated cells (○) or cells treated with 1 \( \mu \)M isoproterenol for 20 min at 37 °C (□) were incubated at 0 °C with 0.25 mg/ml concanavalin A for 20 min, lysed, and layered on a sucrose gradient as described under “Experimental Procedures.” The binding of \([125I] ICYP to receptors in each fraction was determined in the presence (dashed lines) and absence (solid lines) of CGP12177 as described under “Experimental Procedures.” The behavior of \( \beta \)-adrenergic receptors in L cells that express hamster \( \beta \)-receptors (A8) and L cells that express turkey adrenergic receptors (LSV,7, LSV,22) are compared. Similar experiments using clones LSV,11, 13, and 49 gave results similar to those for clone LSV,7 (results not shown).

![Image](image-url)

**Fig. 4.** Effect of exposure to isoproterenol on the capacity of CGP12177 to compete with ICYP for binding. Control cells (○) or cells treated with 1 \( \mu \)M isoproterenol for 20 min at 37 °C (□) were lysed and diluted with binding buffer. Competition binding experiments were performed with 10 pM \([125I] ICYP, and the concentrations of CGP12177 indicated for 2 h at 37 °C. The properties of hamster \( \beta \)-adrenergic receptors expressed in A8 L cells are compared with those of \( \beta \)-adrenergic receptors expressed in L cell clones LSV,7, LSV,11, LSV,22, LSV,30, and LSV,49. Each panel shows a single experiment (each point in triplicate) that is representative of two to four similar experiments.
their receptors became relatively insensitive to competition by CGP12177, as was observed for hamster \( \beta \)-adrenergic receptors in clone A8. Thus, all but one of the LSV clones failed to display endocytosis according to either assay, as is characteristic of avian cells, whereas LSV,22 displayed agonist-induced receptor endocytosis that is commonly observed in mammalian cells.

The ability of recombinant \( \beta \)-adrenergic receptors to undergo down-regulation paralleled their ability to be endocytosed. When L cells that express \( \beta \)-adrenergic receptors were treated for up to 24 h with 1 \( \mu \)M isoproterenol, clones LSV, 7, 10, 11, 13, 30, and 49 showed no loss of receptors (Table III). In contrast, a substantial loss of receptors was observed in clone LSV,22 (57% in 5 h; 78% in 21–24 h). Again, receptors in LSV,22 behaved in a manner characteristic of receptors in mammalian cells, and receptors in the other clones showed characteristics of avian erythrocytes.

**LSV,22 Cells Express a Truncated \( \beta \)-Adrenergic Receptor—**

As an initial probe of the structure of the avian \( \beta \)-adrenergic receptor in L cells, receptors were photoaffinity labeled with \[^{125}\text{I}]\text{ICYP-diazirine and resolved by dodecyl sulfate polyacrylamide gel electrophoresis. As shown in Fig. 5, left, for LSV,7, the recombinant turkey erythrocyte \( \beta \)-adrenergic receptor displayed an apparent \( M_r \) of 45,000. Similar mobility was observed for the receptor expressed in LSV,11 (Fig. 5, right), in several clones of transfected Chinese hamster ovary cells and in transfected COS cells (not shown). \( \beta \)-Adrenergic receptors expressed in LSV,22 cells displayed greater mobility that was consistent with an apparent \( M_r \sim 33,000 \) (Fig. 5). Although the \( \beta \)-adrenergic receptor is known to be sensitive to proteolysis without loss of activity (20), the reduced size of the receptor in LSV,22 does not appear to be caused by proteolysis. Its mobility was constant in several separate preparations, it was not altered by the inclusion of protease inhibitors, and it was observed under conditions where the receptor in other L cells was not proteolyzed.

Controlled proteolysis with trypsin was used to compare the affinity-labeled receptor in LSV,22 cells with the larger receptor in LSV,11 cells. Receptors were labeled with ICYP-diazirine in the presence or absence of propranolol and treated with trypsin under non-denaturing conditions. Because of the low concentration of receptors in the LSV,11 membranes, nonspecific labeling was significant, but the receptor and its products can be identified as those bands whose labeling is blocked by propranolol. The limit digest of the labeled receptors from either clone contained two radioactive fragments of apparent \( M_r \sim 10,000 \) and 20,000, as previously described for digests of receptors isolated from erythrocytes (21). The larger fragment represents spans 1–5 and the connecting loops and the smaller fragment represents spans 6 and 7 and the connecting loop (21). The identical electrophoretic mobilities of the fragments in LSV,11 and 22 indicate that the loss of mass in LSV,22 occurs either at the amino or carboxyl terminus or in the large cytoplasmic loop that connects spans 5 and 6; i.e. those domains in which tryptic proteolysis occurs.

To identify the mutation in the receptor in LSV,22 cells, the receptor DNA was isolated from a preparation of total genomic DNA and sequenced. The region of the cDNA that encodes the cytoplasmic C-terminal domain of the receptor was unaltered from codon 383 through codon 412. At that point, the receptor DNA was isolated from a preparation of total genomic DNA and sequenced.

**TABLE III
Receptor down-regulation**

Values are the means of triplicate determinations in (n) experiments. Some of the binding assays were not performed with saturating concentrations of the radioligand. Therefore, only relative binding is presented.

<table>
<thead>
<tr>
<th>Clone</th>
<th>[^{125}\text{I}]\text{ICYP binding}</th>
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<tbody>
<tr>
<td></td>
<td>( \text{b h} )</td>
</tr>
<tr>
<td>LSV,7</td>
<td>105 (2)</td>
</tr>
<tr>
<td>LSV,10</td>
<td>98 (2)</td>
</tr>
<tr>
<td>LSV,11</td>
<td>105 (1)</td>
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<tr>
<td>LSV,13</td>
<td>101 (1)</td>
</tr>
<tr>
<td>LSV,22</td>
<td>43 (4)</td>
</tr>
<tr>
<td>LSV,30</td>
<td>87 (3)</td>
</tr>
<tr>
<td>LSV,49</td>
<td>120 (1)</td>
</tr>
</tbody>
</table>

![Fig. 5](image-url) **FIG. 5.** Photoaffinity labeling of recombinant \( \beta \)-adrenergic receptors in L cells. Left, a particulate fraction from untransfected L cells (L), LSV,22 (22), or LSV,7 (7) was labeled with \[^{125}\text{I}]\text{ICYP-diazirine, dissolved in} dodecyl sulfate, and electrophoresed on a 10% polyacrylamide gel. The concentration of label was 64 \( \mu \)M and was used in the presence or absence of 5 \( \mu \)M (–)-propranolol (Prop). The molecular sizes of marker proteins (kDa) are shown at right (T, top of gel). Right, a similar experiment in which receptors from LSV,11 and LSV,22 are compared. Some of the membranes were treated with trypsin prior to solubilization, as shown. Arrows indicate the locations of the untreated receptors and the locations of the tryptic fragments. Because of the low concentration of receptors in the membranes from LSV,11, proteins that were labeled nonspecifically (in the presence of propranolol) are also prominent. This includes a protein with mobility just greater than that of the receptor.
point, previously unidentified DNA sequence was found for more than 150 base pairs:

Wild type

\[
\begin{align*}
\text{Wild type} & \quad 416 \\
\text{GAGGCAACATGGTCCGGATCGAGTCAAGTGGGA} & \quad 425 \\
\text{LSV,22} & \\
\end{align*}
\]

Such a sequence was probably caused by a recombination event when the receptor cDNA was incorporated into the L cell genome. The predicted amino acid sequence of the mutant \( \beta \)-adrenergic receptor in LSV,22 cells is normal through Glu\(^{412} \). The carboxy terminus of the mutant receptor is Ser\(^{413} \) shorter by 59 residues than the wild type. It is a striking coincidence that the length of the mutant receptor is exactly that of the \( \beta \)-adrenergic receptor (31) when the two are aligned according to Varden et al. (16).

**DISCUSSION**

The data presented here indicate that four distinctive properties of the \( \beta \)-adrenergic receptor of turkey erythrocytes are determined by the structure of the receptor itself rather than by other components of the erythrocyte membrane. These properties are: 1) a characteristic selectivity for \( \beta \)-adrenergic ligands similar to that of mammalian \( \beta \)-adrenergic receptors except for a few diagnostic antagonists; 2) negligible formation of the agonist-receptor-Gs complex that is commonly observed as guanine nucleotide-sensitive high affinity binding of agonists; 3) lack of agonist-induced receptor endocytosis; and 4) lack of agonist-induced receptor loss (down-regulation). With the exception of one interesting variant clone, LSV,22, recombinant receptors expressed in murine L cells displayed typical avian behavior in each respect. As expected, both hamster and human \( \beta \)-adrenergic receptors exhibit GTP-sensitive, high affinity agonist binding and undergo agonist induced internalization and down regulation when expressed in mammalian cells (34-36). Furthermore, it is unlikely that the lack of endocytosis and down-regulation in avian erythrocytes might simply reflect their inability to perform endocytosis because \( \beta \)-adrenergic receptors are also not endocytosed in turkey reticulocytes (29), which have been shown to endocytose transferrin receptors (32, 33).

The variant receptor in clone LSV,22 is of special interest in defining the structural elements in the receptor that mediate endocytosis, down-regulation, and formation of the receptor-Gs complex. The selectivity for \( \beta \)-adrenergic drugs of the receptor in LSV,22 cells is distinctively avian and is not distinguishable from receptors in turkey erythrocytes or in the other clones examined. It is, therefore, a product of the transfected cDNA rather than of a spontaneously activated L cell gene. However, its ability to display GTP-sensitive, high affinity agonist binding and undergo agonist-induced internalization and down-regulation are distinctly mammalian. Thus, the receptor in LSV,22 cells has acquired novel functions characteristic of mammalian receptors, and the nature of the alteration in its structure may define the structural determinants of these processes.

The most obvious difference between the mutant \( \beta \)-adrenergic receptor expressed in LSV,22 cells and the receptors expressed in other mammalian cells is the net loss of 59 amino acid residues in the cytoplasmic carboxyl-terminal domain. This domain has already been implicated in at least some modes of desensitization of mammalian receptors (34, 35).

\[ \text{The } \beta \text{-adrenergic receptor can tolerate sizable deletions in this domain without loss of acute function (30, 35-37). Be-} \]

because the wild type avian receptors have a much larger C-terminal domain than do mammalian receptors (16), this region in the mutant receptor is actually about the same size as that of its mammalian counterparts.

One can entertain the idea that the "extra" avian sequence serves to restrict proteins involved in triggering internalization and down-regulation from access to target sites in the more proximal portion of this domain and thereby prevents these reactions. Consequently, loss of the extra segment could confer mammalian properties on a truncated \( \beta \)-adrenergic receptor. Possible targets might include the 14 serine and threonine residues between positions 382 and 422 (11 in the same region of the hamster receptor). It is also possible, but less intuitively likely, that the 12 new C-terminal amino acid residues in the mutant receptors contribute to its phenotype, and this possibility will be tested genetically.

The concurrent appearance of GTP-sensitive high affinity agonist binding and of receptor endocytosis and down-regulation in the mutant receptor is surprising. The former phenomenon is thought to reflect interaction with Gs (9, 10) and the latter phenomena have been assumed to indicate interaction with kinases, the cytoskeleton, or other cellular proteins (4). These interactions may now be found to be mechanistically linked. Alternatively, different regions in the carboxyl terminus may be independently responsible for the two events. Biochemical analysis of the receptor from LSV,22 and related mutant receptors should clarify these questions.

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**REFERENCES**

An Endocytosis-competent Avian β-Adrenergic Receptor