A new, hydrophilic β-adrenergic receptor radioligand, (±)-[3H]CGP-12177 (4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on hydrochloride), was synthesized and radiolabeled to 40 Ci/mmol. The nonspecific binding of this compound to turkey erythrocyte ghosts and C6 glioma cell membranes was less than 5% of the total binding at five times the appropriate $K_D$. Binding assays of intact C6 glioma cells also showed low nonspecific binding, less than 20% of the total binding at five times the appropriate $K_D$.

The affinities of the antagonists (−) and (+)-propranolol as well as of the agonist (−)-isoproterenol were examined by their potency to displace various radioligands from intact C6 glioma cell and membrane preparations. With membrane preparations, both [3H]CGP-12177 and [3H]dihydroalprenolol (DHA) were displaced stereospecifically by the antagonists (−) and (+)-propranolol and the agonist (−)-isoproterenol. With whole cells, [3H]CGP-12177 and [3H]DHA behaved differently. [3H]DHA and [3H]carazolol could be stereospecifically displaced by antagonists but only partially displaced by agonists, while [3H]CGP-12177 could be completely displaced by both antagonists and agonists as in membranes. In contrast to [3H]CGP-12177, the lipophilic ligand [3H]DHA is actually taken up by cells.

The inability of agonists to displace lipophilic radioligands from receptors on intact cells may not be due to a low affinity of agonists for receptors on cells, but to an agonist-induced change in the receptors which renders them inaccessible to hydrophilic agonists and antagonists. This change is likely to be their internalization into the cell.

Determination of the number of β-adrenergic receptors in a membrane preparation became possible with the introduction of high affinity β-adrenergic antagonists which were radiolabeled to a high specific radioactivity, [3H]DHA (Lefkowitz et al., 1974) and [3H]IHYP (Aurbach et al., 1974).

These ligands bind saturably with respect to time and concentration, they are displaced by β-adrenergic agents in the order of potency that corresponds to the agents' biological activity, and their binding is reversible. Recently, three more β-adrenergic radioligands, (±)-[3H]carazolol (Innis et al., 1979), (−)-[3H]iodopindolol (Barovsky and Brooker, 1980), and (±)-[125I]iodocyanopindolol (Engel et al., 1981) have been introduced. All of these compounds are hydrophobic and stick to glass or polystyrene, but give reasonably low amounts of nonspecific binding with membrane preparations.

These ligands have been used to assay β-adrenergic receptors on intact cells (Insel and Stoolman, 1978; Schmitt and Pochet, 1977; Pittman and Molinoff, 1980; and Barovsky and Brooker, 1980), and in general, the nonspecific binding represented more than 40% of the total counts bound at two times the $K_D$ of the ligand. This high nonspecific background can often be reduced by taking advantage of the fact that the ligand-receptor complex has a relatively long half-life, and therefore a postincubation rinse will eliminate the bulk of the nonspecific counts while retaining the majority of specifically bound counts. When the $K_D$ values for adrenergic ligands were determined for intact cells, the $K_D$ values for antagonists were similar to their values in membrane preparations, but the $K_D$ values for agonists were over 2 orders of magnitude higher than their values in membrane preparations (Terasaki and Brooker, 1978; Insel and Stoolman, 1978). In the presence of levels of GTP found intracellularly, the $K_D$ values of agonists in membrane preparations were shifted about 10-fold higher (Lefkowitz et al., 1976; Maguire et al., 1976a), but even this left a factor of 10 or more between the affinities of agonists for receptors in membrane preparations and in intact cells.

Two recent papers have indicated that following isoproterenol stimulation β-adrenergic receptors were internalized (Chuang and Costa, 1979; Harden et al., 1980). Although the above-mentioned ligands may give adequate information about receptors on unstimulated cells, it is probable that the lipophilic probes dissolve in the plasma membrane, distribute internally, and can therefore interact with internalized receptors. The fact that often more binding can be displaced by propranolol than by isoproterenol is consistent with this hypothesis (Bylund, 1978; Stone and U'Prichard, 1981).

The use of a hydrophilic ligand can circumvent the problem of ligand-binding to internalized receptors (Shoyab and Todd, 1980). CGP-12177 is such a hydrophilic β-adrenergic antagonist. The results reported here show that [3H]CGP-12177 is suitable for assaying β-adrenergic receptors in intact cells. It is probable that it measures only cell surface receptors.

We report here that [3H]CGP-12177 gives nearly the same $K_D$ values for agonists in membrane preparations and intact cells. Agonist-induced endocytosis may account for the difference between apparent agonist affinities determined with lipophilic and hydrophilic radioligands.

The abbreviations used are: [3H]DHA, (−)-[3H]dihydroalprenolol; [2H]IHYP, (±)-[2H]iodohydroxybenzylpindolol; [3H]CGP-12177, (±)-[3H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on hydrochloride; $K_{on}$, the concentration giving a half-maximal response; $IC_{50}$, the concentration necessary to reduce a response by 50%; DMEM, Dulbecco's modified Eagle's medium; DMEM-Hepes, DMEM without NaHCO$_3$ but containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, and 0.036 M NaCl instead; PBS, phosphate-buffered saline.

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A HYDROPHILIC β-ADRENERGIC RECEPTOR RADIOLIGAND REVEALS HIGH AFFINITY BINDING OF AGONISTS TO INTACT CELLS*

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Experimental Procedures

(±)-Alprenolol was obtained from AB Hassel, Mölndal, Sweden; phenoxyamine from Ciba-Geigy Limited, Basel, Switzerland; and (+)- and (−)-propranolol from Ayerst Laboratories, New York. (−)-Isoproterenol bitartrate and (−)-norepinephrine were obtained from Sigma. All other materials used were of reagent grade. [3H]DHA and [3H]iodocyanopindolol were obtained from the Radiochemical Centre (Amersham, England) and the specific radioactivity of [3H]DHA was checked by bioassay against cold DHA. [3H]Carazolol was obtained from New England Nuclear.

The structure of CGP-12177 is shown in Fig. 1; its synthesis has been described (German Patent 2700193). Tritiation was carried out by first brominating the compound, and then exchanging bromine with 3H details of the synthesis and radiolabeling are available from the authors.

Two crude membrane preparations rich in β-adrenergic receptors, turkey erythrocyte ghosts (Gill, 1976) and C6 rat glioma cell membranes (Maguire et al., 1976b) were used. Membrane-binding assays were performed after resuspending the membranes with 10 strokes of a tight-fitting Dounce homogenizer in 5 mM Hepes, 1 mM MgSO4, pH 8 buffer, at a concentration of 0.25–0.5 mg of protein/ml. The membranes were incubated for 20 min at 30 °C with the radioligands added simultaneously with any competing drug. At the end of incubation, samples were diluted 20-fold with ice-cold termination buffer (10 mM potassium phosphate, pH 7.4, 1 mM MgSO4) and filtered through Whatman GF/C glass filter. The filters were washed twice with 10 ml of 0 °C termination buffer, and 10 ml of Instagel (Packard Instrument Co., Downers Grove, IL) were added while the filters were still wet. The vials were gently shaken for 30 min, and then counted at 44% efficiency in a Packard scintillation counter. Drying the filters before counting reduces the counting efficiency, because [3H]CGP-12177 is so hydrophilic that once it is dried in proteinaceous solutions it does not dissolve in toluene-based scintillators (Staehelin and Simons, 1981). Nonspecific binding was defined as the binding observed in the presence of 1 μM (−)-propranolol. C6 glioma cells (Benda et al., 1968) were grown in DMEM (Gibco, Grand Island, NY) containing 5% fetal calf serum in 10-cm plastic dishes (Corning No. 25020, Corning, NY) to a density of 25 × 106 cells/dish (Drummond et al., 1977). They were loosened from the plates by a 2-min treatment with 0.02% EDTA in phosphate-buffered saline, removed by vigorous pipetting in DMEM-Hepes and suspended in this buffer.

Incubations were carried out in a total volume of 0.5 ml containing 10⁶ glioma cells. Filtration was done as with membranes.

cAMP production in C6 glioma cells was measured by aspirating the medium from confluent cultures, replacing it with 1 ml of DMEM-Hepes medium containing 10⁻³ M IBMX and allowing the cells to come to a new steady state for 30 min at 21 °C. Fifty μl of CGP-12177 were then added and 10 more min were allowed for binding to come to equilibrium; then 50 μl of 10⁻⁶ M (−)-isoproterenol in 0.02% ascorbic acid were added. cAMP was allowed to accumulate for 10 min; then the medium was aspirated and 3 ml of 5% trichloroacetic acid was added and left for at least 1 h. Aliquots of the trichloroacetic acid extracts were assayed for cAMP using the cAMP assay kit from the Radiochemical Centre.

Protein was determined by a modified Lowry procedure (Peterson, 1977).

All points in the figures represent the average of triplicate values unless otherwise noted. Equilibrium KD values were determined by Scatchard analysis (Scatchard, 1946).

Dissociation rate constants were determined by incubating [3H]CGP-12177 with the membranes under assay conditions at about 10 × KD for 20 min at 30 °C, then adding a 100-fold excess of unlabeled ligand and measuring bound radioactivity by filtration at various intervals. Association rate constants were determined by incubating 0.1 nm [3H]CGP-12177 with the membranes under assay conditions, stopping the association by filtration at 15, 30, 45, and 60 s. The data were linearized using the following equation (Riggs et al., 1970)

\[ \frac{1}{(R-L)}\ln \frac{L(R-R-L)}{R(L-R-L)} = k_t t \]

where R = total receptor, L = total ligand, R-L = receptor ligand complex at time t, k_t = the bimolecular association rate constant, and t is the time of association.

Results

The chemical structure of CGP-12177 (Fig. 1) is analogous to other well-characterized β-adrenergic antagonists. The PBS:octanol partitioning ratios for various β-adrenergic ligands were determined (Table I).

In Vitro β-Adrenergic Properties of CGP-12177—Previous studies (German Patent 2700193) had shown that CGP-12177 was a potent β-adrenergic blocker in vivo with no detectable sympathomimetic effects unless it was used in extremely high doses. The ability of CGP-12177 to block a typical β-adrenergic response in vitro, the isoproterenol-stimulated synthesis of cAMP in C6 rat glioma cells, was studied. Briefly, CGP-12177 was added to culture dishes for 10 min at 21 ºC, and then 100 nm (−)-isoproterenol was added to stimulate cAMP production for 10 min. The top line in Fig. 1 shows that cAMP production was blocked by CGP-12177 with an IC₅₀ of 5 nm, but that the inhibition never reached 100%, and in fact cAMP levels rose between CGP-12177 concentrations of 0.1 and 10 μM. The agonist activity on C6 glioma cells of CGP-12177 alone is represented by the bottom curve in Fig. 1. This compound is a partial agonist (15% of the maximal (−)-isoproterenol-induced cAMP response), with a Kₐ of 200 nm. Since the Kₐ of (−)-isoproterenol for C6 glioma cells is 20 nm (Maguire et al., 1976b) and since the IC₅₀ in this experiment was one-twentieth of the isoproterenol concentration used, a Kₐ of about 1 nm can be estimated. The partial agonist activity, however, appears only with a Kₐ of 200 nm. Hydroxybenzyl-
pindolol is also a partial agonist at high concentrations (Drummond et al., 1977).

**Binding Studies with Crude Membrane Preparations—** Preliminary studies with turkey erythrocyte ghosts at 30°C showed that binding of 0.1 nM [³H]CGP-12177 was apparently complete in 10 min and that the half-time for receptor-ligand complex dissociation was about 4 min. Therefore, an incubation time of 20 min was chosen to obtain the binding curves shown in Fig. 2. [³H]DHA was used in the same experiment for comparison. It can be seen that [³H]CGP-12177 binds to the receptor with a $K_D$ of 0.3 ± 0.1 nM (average of three separate experiments). The $K_D$ of [³H]DHA was 0.6 nM.

The number of binding sites measured with [³H]CGP-12177 was slightly lower than the number shown by [³H]DHA in this experiment. In three experiments, [³H]CGP-12177 showed an average of 5% fewer binding sites than did [³H]DHA. This is within the limit of accuracy of the experiments, but experiments to be reported later in this paper suggest that in fact there may be some small amounts of nonspecific binding at high levels of [³H]DHA. The fact that [³H]CGP-12177 and [³H]DHA measured the same number of receptors suggests that they were measuring the same receptors. Both ligands displayed acceptably low nonspecific binding at 5 × $K_D$, a level at which 83% of the receptors are theoretically bound (Fig. 3, A and C).

Experiments with C6 glioma cell membranes gave $K_D$ values of 0.2 ± 0.1 and 0.5 ± 0.1 nM for [³H]CGP-12177 and [³H]DHA, respectively. The half-life of the receptor-ligand complex with turkey erythrocyte ghosts was 4 min at 30°C and that of C6 glioma cell membranes was 25 min at 37°C. This latter value is about 15 times longer than that of the receptor-[³H]DHA complex. The association rate, however, as determined by short time incubations described under “Experimental Procedures” was also five times slower, i.e. 0.2 × 10⁶ M⁻¹ min⁻¹ versus a value of 1 for [³H]DHA (average of three experiments). Since $K_D$ is defined as $K_{-1}/K_{+1}$, it is understandable that [³H]CGP-12177 has a three times lower $K_D$ than [³H]DHA. But it is important to know that, in view of the slower on-rate, longer times of incubation are necessary to reach equilibrium.

**Binding Studies with Intact Cells—** Fig. 4 shows the binding of [³H]CGP-12177 and [³H]DHA to intact C6 glioma cells. The average value of $K_D$ for [³H]CGP-12177 from five such experiments was 0.3 ± 0.2 nM. It can be seen that the nonspecific counts constitute only 10% of the total bound counts at five times the $K_D$ for [³H]CGP-12177 (Fig. 3B), while the nonspecific binding of [³H]DHA was always greater than the specific binding (Fig. 3D). The number of binding sites per cell was calculated to be 8500, close to the 9400 sites/cell found by Dibner et al. (1981) using [¹²⁵I]iodohydroxybenzylpindolol, and about the same as given by [³H]DHA binding on the same cells. (The high error associated with the [³H]DHA measurements precluded an accurate estimate of $B_{max}$.)

A number of different cell types were tested to determine whether this low nonspecific binding was a general phenomenon, including the WEHI 7 mouse lymphoma cell line (Harris et al., 1973) and mouse macrophages, B cells, T cells and cortisone-resistant T cells (all four lymphocyte subpopulations were obtained from Dr. R. Papiol, Friedrich Miescher-Institut). Even with the last subpopulation, which displayed 150 receptors/cell, the nonspecific binding was only 40% of the total binding at 5 × $K_D$. It is probably the hydrophilicity of CGP-12177 which results in low nonspecific binding.

The lipophilicity of [³H]DHA suggests why high nonspecific binding was observed in cell assays of β-adrenergic receptors. It was still necessary to explain why such low nonspecific binding was observed with lipophilic ligands when assaying...
membrane preparations, in contrast to the high nonspecific binding observed with intact cells.

Cellular Uptake of [3H]DHA—The low nonspecific binding of [3H]DHA to membranes could be due either to the absence of any binding or to its rapid removal by the dilution with washing buffer or the subsequent washing of the filters. The nonspecific binding of a cell suspension and a cell homogenate was therefore measured by centrifugation as well as by filtration at various times after dilution with washing buffer. C6 cells and a homogenate from the same number of cells were allowed to equilibrate with 1 nM [3H]DHA in the presence or absence of 1 μM (-)-propranolol. These suspensions were then centrifuged and the amount of radioactivity in the pellets was determined by difference in the medium before and after centrifugation or by the radioactivity in the pellet, which gave the same results within experimental error.

With [3H]CGP-12177, the nonspecific binding in the pellets was less than 20% of the specific binding. The nonspecific binding of [3H]DHA, in contrast, was two to three times higher than the specific binding in the pellets of both cells and homogenates. These results are shown as zero values in Fig. 5 and indicate high initial nonspecific binding for both preparations. Aliquots of the same suspensions were also diluted with 20 volumes of cold buffer and filtered after various times. Cell homogenates, which initially bind almost as much [3H]DHA nonspecifically as intact cells, release it almost immediately upon dilution (Fig. 5B, Δ). Intact cells release only about two-thirds of the nonspecifically bound radioactivity within 10 min, whereas about one-third remains rather stably bound (Fig. 5B, ○). This is to be expected if [3H]DHA binds not only to plasma membranes but to membranes of inner compartments as well. Evidence that the plasma membrane is an important permeability barrier for the remaining [3H]DHA comes from the finding that cells diluted with an isotonic buffer release the nonspecifically bound [3H]DHA only slowly (Fig. 5B, ○), whereas cells in a hypotonic buffer (which causes swelling and increases cell permeability) release their nonspecifically bound [3H]DHA much more rapidly (Fig. 5B, □).

This result indicates that [3H]DHA is not only bound to plasma membranes but to inner compartments of the cell as well. It also yielded a convenient method to study binding of [3H]DHA to intact cells since the specifically bound [3H]DHA shows only a slight decrease during 30 min (Fig. 5A). Thus, dilution with a hypotonic buffer 30 min prior to filtration was sufficient to reduce the nonspecific binding with only a 10-15% loss in specific binding. Having established this methodology for measuring the binding of lipophilic ligands to intact cells, the affinity of various nonlabeled agonists and antagonists was studied.

Competition Studies on Membranes—On membranes from C6 glioma cells, both [3H]DHA and [3H]CGP-12177 behave as typical β-adrenergic ligands. Both can be displaced stereospecifically by (-)- and (+)-propranolol and also by (-)-isoproterenol (Fig. 6). That they bind to the same receptors is
in the presence of guanyl nucleotides. Thus, at least two- thirds of the receptors are in the R(L) state as would be expected in the presence of a sufficiently high intracellular GTP concentration (Lesznowitz et al., 1981). In contrast, [3H] DHA and [3H]carazolol can only be displaced at extremely high isoproterenol concentrations. While isoproterenol is equipotent with (+)-propranolol in displacing [3H] CGP-12177, [3H]DHA and [3H]carazolol are displaced only 10-20% at the isoproterenol concentrations corresponding to the IC50 for (+)-propranolol and even 100 µM isoproterenol displaces less than 90% of the ligand (Fig. 7). Since the reason for the difference could be that equilibrium was not reached in 15 min, longer incubations were carried out which gave the same results.

These results are in agreement with those of other authors who have reported previously that agonists are much less potent in displacing lipophilic ligands from intact cells than from membranes (Terasaki and Brooker, 1978; Insel and Stoolman, 1978). Fig. 7 shows that this is not the case for the hydrophilic ligand [3H] CGP-12177. This discrepancy is not only seen in C6 glioma cells since nearly identical data were obtained with S49 and WEHI 7 lymphoma cells (data not shown). Likewise, it is not restricted to isoproterenol since [3H]CGP-12177 could also be displaced by (-)-epinephrine and (-)-norpinephrine. The latter were 6 and 15 times less active than isoproterenol in agreement with their potency ratio in C6 membranes (Maguire et al., 1976b). Lastly, the binding of [3H]DHA in the presence of isoproterenol cannot be attributed to a change in dissociation rate since the half-lives of the receptor-ligand complex from cells incubated with [3H]DHA in the presence and absence of isoproterenol were very similar (3.8 and 5.1 min, respectively; data not shown). The discrepancy obtained with different ligands can be explained, however, if in addition to R(H) and R(L) receptors present on the cell surface a third class of receptors is postulated in intact cells which has access to neither isoproterenol nor [3H]CGP-12177 but only to [3H]DHA. The existence of such a third class of receptors is suggested by the experiments of Harden et al. (1980) who demonstrated an isoproterenol-induced shift of about half the receptors of intact astrocytoma cells from the plasma membrane to a small vesicle compartment which the authors considered to be endocytotic vesicles. How the existence of receptors in such endocytotic vesicles could explain our experimental data is discussed below.

**DISCUSSION**

[3H] CGP-12177 has a chemical structure similar to those of other β-adrenergic ligands (Fig. 1). The side chain is of the N- tertiary butyl type, which is reported to be of higher affinity than corresponding N-isopropyl analogues (Kaumann et al., 1979).

Secondly, CGP-12177 blocks both in vivo and in vitro responses to the β-adrenergic agonist isoproterenol. Under the conditions used in this laboratory, CGP-12177 displayed a KD of about 1 nM for blocking isoproterenol-induced cAMP response in rat C6 glioma cells. This is in reasonable agreement with an observed equilibrium KD of 0.3 nM for binding to intact C6 cells. The compound also displayed a weak partial agonist effect (15% of the isoproterenol-induced cAMP response) with a Kact of 200 nM, over 2 orders of magnitude higher than the KD. This partial agonist activity is probably due to the imine >N—H moiety which is also present on pindolol; both imines probably weakly mimic the catechol —O—H moieties of β-adrenergic agonists.

Finally, [3H]CGP-12177 displays the pharmacology expected on a β-adrenergic receptor radioligand. Comparison of [3H]CGP-12177 with the widely used [3H]DHA was undertaken using two well characterized membrane preparations,
turkey erythrocyte ghosts and rat C6 glioma cells. [\(^{3}H\)]CGP-12177 bound approximately 95% of the sites bound by [\(^{3}H\)]DHA. This is within the level of accuracy of these experiments, but in displacement studies similar to those shown in Fig. 4, dihydroalpranolol displaced [\(^{3}H\)]CGP-12177 completely, whereas CGP-12177 always left a small amount of [\(^{3}H\)]DHA bound (data not shown). This led us to believe that perhaps [\(^{3}H\)]DHA was binding to some small class of receptors not accessible to [\(^{3}H\)]CGP-12177, or to nonspecific sites (Bylund, 1978; Stone and U’Prichard, 1981). [\(^{3}H\)]CGP-12177 bound both types of receptors three times as strongly as did [\(^{3}H\)]DHA.

Competition binding experiments showed that [\(^{3}H\)]CGP-12177 was displaced by a series of \(\beta\)-adrenergic ligands in a rank order of potency corresponding to their known potencies on \(\beta\)-adrenergic systems, and especially that (-)-propranolol was 100 times more effective than (+)-propranolol in displacing [\(^{3}H\)]DHA bound (data not shown). This led us to believe that perhaps [\(^{3}H\)]DHA was binding to some small class of receptors not accessible to [\(^{3}H\)]CGP-12177, or to nonspecific sites (Bylund, 1978; Stone and U’Prichard, 1981). [\(^{3}H\)]CGP-12177 bound both types of receptors three times as strongly as did [\(^{3}H\)]DHA.

Isoproterenol is hydrophilic (Table I), shows low nonspecific binding to cells (25% of total binding at 2.7 \(\times\) 10\(^{-7}\) M; Malchoff and Mariotti, 1978), and probably remains extracellular. We propose that when radioligand, agonist, and cells are incubated together in a competitive binding experiment, agonist-induced endocytosis of a fraction of the receptors occurs. The internalized receptors thereby become inaccessible to hydrophilic ligands such as isoproterenol or [\(^{3}H\)]CGP-12177 but remain accessible to lipophilic ligands such as [\(^{3}H\)]DHA, [\(^{3}H\])carazolol, and \([^{125}I]\)IYP. Thus, the binding of all [\(^{3}H\)]CGP-12177 can be blocked by isoproterenol, but both are outside the cell. [\(^{3}H\)]DHA would, in contrast, bind to both populations of receptors but be displaced by isoproterenol from only one; the expected biphasic curves are shown in Fig. 7. Additionally, it can be predicted that since endocytosis is a temperature-dependent process (Maxfield et al., 1978), cells incubated at 0 °C should show the same high affinity for agonists as shown by membranes, even with lipophilic radioligands. This has already been demonstrated (Isotel and Sanda, 1979).

Thus, the model offers a convenient explanation for the finding that the receptors in intact cells have a much higher apparent \(K_d\) for agonists when measured with lipophilic radioligands than receptors in membranes. By our model, the receptors do not have a lower affinity in cells, but rather radioligand binding can no longer be displaced because the radioligand is internalized while the agonist remains external. With a hydrophilic ligand that is not taken up by cells, the discrepancy disappears.

Pittman and Molinoff (1980) have shown recently that although the binding of \([^{125}I]\)IYP to the \(\beta\)-adrenergic receptor in mouse L cells is initially blocked by isoproterenol, the receptor is quickly converted to a state where isoproterenol does not hinder \([^{125}I]\)IYP binding. Since this radioligand is also lipophilic and shows similarly high nonspecific binding as the other lipophilic radioligands, these findings are also consistent with the internalization of receptors.

In conclusion, [\(^{3}H\)]CGP-12177 is a hydrophilic \(\beta\)-adrenergic receptor ligand which gives low nonspecific binding with intact cells, and which probably binds only to cell surface receptors. Using this hydrophilic radioligand, cellular \(\beta\)-adrenergic receptors were found to have nearly the same high affinity for isoproterenol as receptors in membrane preparations. The different results obtained with lipophilic radioligands can be explained if it is assumed that receptors are internalized and therefore become inaccessible to hydrophilic agonists.

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