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Trapping of the β-adrenergic receptor in the hormone-induced state

(receptor response/receptor locking/detergent effect on receptor/receptor-induced fit/agonist–antagonist difference)

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ABSTRACT **Isoproterenol and other agonists readily dissociate from the β-adrenergic receptor in turkey erythrocyte membranes. However, when a low concentration of deoxycholate is added, the receptor locks the prebound agonist; i.e., the rate of dissociation of the prebound agonist decreases drastically. The dissociation of prebound antagonists is slightly increased by deoxycholate. Locking, which is thus agonist specific, occurs in the cold, is reversed when detergent is removed from the membranes, and appears not to require the guanyl nucleotide binding protein of the adenylate cyclase system. It is suggested that this induced fit of a receptor to an agonist represents the specific conformational response that normally propagates in the receptor molecule in its interaction with the next component along the pathway of signal transmission.**

It is generally accepted that both the agonist and the antagonist bind in a highly specific manner to the hormone or neurotransmitter receptor. Since only the agonist initiates a response, its interaction with the receptor must differ in some important details from the interaction with an antagonist. It has, however, been difficult to characterize this difference because the response can rarely be measured at the level of the receptor itself (1). For the many receptors that activate adenylate cyclase, a hormone (H) response requires the receptor (R) and the guanyl nucleotide binding protein (G) (2–8) as described below:

$$(i)\ HR + G \rightarrow HRG$$
$$(ii)\ HRG + GTP \rightarrow HR + G_{GTP}.$$

At a subsequent step, G_{GTP} activates the catalytic unit of the enzyme but this is irrelevant to the present work (6). Binding studies indicate that the affinity of the receptor for the agonist is relatively high in the absence of guanyl nucleotides when the HRG complex is formed (step *i*) and that it shifts to a lower value when GTP is added and HR again predominates (step *ii*) (9–11). Such binding studies give useful information about the steady-state statistical average of all the molecules in the sample, but it is not clear how many different agonist–receptor complexes exist, what are their relative amounts, and what are the specific properties of each.

Recently, we trapped the putative HRG complex by alkylation of a specific SH group that is probably located in the G component (12). The agonist was found locked in the stabilized complex. The findings suggested that the normal formation of the activated HRG intermediate involves transient locking of agonist in the receptor. The term "locking" was introduced to describe the transition of a hormone-occupied receptor from a relatively open conformation, showing a readily measurable dissociation of the ligand, to a closed conformation with little, if any, measurable dissociation. This is in contrast to antagonists, some of which have an extremely high affinity, which, however, changes little under various experimental conditions (13, 14). The effect of SH alkylation in the presence of the agonist has been studied with β-adrenergic receptors of different types of cells (15). It is therefore apparent that locking of the agonist in the receptor is not unique to the turkey erythrocyte membranes used in our study.

In the above discussed systems, both locking and high-affinity binding sensitive to GTP are dependent on the induced fit interaction (16) of the HR component with the G component (step *i*). It is therefore difficult to analyze which properties of the locked state should be ascribed to the receptor and which are due to its interaction with the G component. Our aim is to study agonist action at the level of the receptor itself; here, we report on the reversible specific locking of agonist in the β-adrenergic receptor that is produced by low concentrations of deoxycholate (DOC) and appears not to require the G component.

MATERIALS AND METHODS

Turkey Erythrocyte Membranes. These were prepared as described (17). For some experiments, the purified membranes were further treated at pH 11.5 (6, 18), which inactivates the G and C components and removes 50% of the membrane protein while retaining 80–100% of the receptor (referred to as pH 11.5 membranes). As a result, the amount of β-adrenergic receptor and the amount of [^{3}H]isoproterenol locked in the receptor per mg of membrane protein were twice as high after pH 11.5 treatment.

Assay of [^{3}H]Isoproterenol Locking in the Receptor. All manipulations and incubations were carried out at 2 ± 2°C unless otherwise noted. Systems, in 0.2 ml, contained 1 mg of native membranes or 0.5 mg of pH 11.5 membranes in 0.6 μM [^{3}H]-isoproterenol/10 mM 4-morpholinepropanesulfonate buffer, pH 7.5/1 mM $MgCl_2$/1 mM ascorbic acid/1 mM catechol.

Identical mixtures as above, serving for calculation of nonspecific binding, received 60 μM isoproterenol prior to addition of the labeled agonist. In some experiments, the antagonist cyanopindolol (19), 0.3 μM, served to measure nonspecific binding. After 15 min, the systems received 20 μl of incubation medium containing 10 mg of DOC/ml. After an additional 10 min, the systems were diluted 1:10 with incubation medium containing 1 mg of DOC/ml. Four minutes later, the tubes were centrifuged for 10 min at 29,000 × *g*. The membrane pellet was suspended in 2 ml of incubation medium containing 1 mg of DOC/ml and 0.3 mM isoproterenol. Unless otherwise noted, systems were incubated at 2°C for 30 min to allow for any exchange of bound [^{3}H]isoproterenol with the large excess of unlabeled agonist. At the end of incubation, duplicate aliquots of 0.2 and 0.4 ml were placed on GF/C filters previously wetted

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Abbreviations: DOC, deoxycholate; GTP[S], guanosine 5'-[γ-thio]triphosphate.

with cold filter wash medium containing (mM) potassium phosphate (pH 8.0), 20; $MgSO_4$, 1; ascorbate, 1; catechol, 1; isoproterenol, 0.3; and DOC, 1 mg/ml. After filtration of the samples at 4°C, the filters were washed with 25 ml of wash medium. Filter-bound radioactivity was determined, and specific binding was calculated by subtracting nonspecific binding from total binding. Nonspecific binding was 15–35% of total binding, depending on the batch of [^{3}H]isoproterenol.

Reagents. [^{3}H]Isoproterenol at about 10 Ci/mmol (1 Ci = 3.7×10^{10} Bq) was obtained from New England Nuclear and Amersham and kept below pH 4. [^{3}H]Alprenolol (32 Ci/mmol) and [^{3}H]propranolol (23 Ci/mmol) were from Amersham. Cyanopindolol was a gift from G. Engel (Sandoz). All other chemicals were of analytical grade.

Accuracy and Reproducibility. Each experiment was repeated at least twice but in most instances several times, with or without minor variations that did not materially affect the results. Within each experiment, the different systems were run in duplicate. Two aliquots, 0.2 and 0.4 ml, each in duplicate, were taken for assay from each system. The bars in the histograms indicate the deviation from the mean in duplicate systems.

RESULTS

Incubation of turkey erythrocyte membranes with [^{3}H]isoproterenol in aqueous medium led to some specific binding of the hormone to the β-adrenergic receptor. However, the hormone dissociated from the membranes fairly rapidly, even in the cold, when the membranes were sedimented and resuspended in excess unlabeled isoproterenol (Fig. 1). In contrast, when membranes containing bound [^{3}H]isoproterenol were washed and resuspended in the presence of a low concentration of DOC (solubilizing <10% of the protein), the hormone became locked in the receptor. There was little, if any, dissociation of hormone from the receptor during the entire 2 hr of the experiment. No less striking was the finding that the specific competitive β-adrenergic receptor antagonists dihydroalprenolol and propranolol were not locked in the receptor by addition of DOC. In fact, these blocking agents dissociated somewhat faster in presence of the detergent. Dissociation was not due to receptor inactivation. When after 60 min the DOC was removed, [^{3}H]-alprenolol was rebound efficiently. It should be noted that 0 time in Fig. 1 is defined as the time after removal of unbound ligand and addition of chase isoproterenol. Therefore, this time point does not necessarily represent the maximal amount of ligand bound. Experiments were carried out at 2°C because the receptor apparently does not interact with the G component in the cold (21, 22), the affinity of isoproterenol is higher than at elevated temperature (14), and the effect of detergent is relatively mild. Incubation of the membranes with [^{3}H]isoproterenol at 37°C prior to transfer to the cold had no effect on subsequent locking. [^{3}H]Norepinephrine could also be specifically locked but nonspecific binding was considerably higher than with [^{3}H]isoproterenol (data not shown). The concentration of DOC as well as its amount relative to the amount of membrane protein seemed to be fairly critical. Locking became fully effective at 1 mg of DOC/ml and 1 mg of DOC/0.5 mg of membrane protein. The experiments worked equally well with three kinds of preparations of turkey erythrocyte membranes, hypotonically lysed cells that still retain the nucleus, purified membranes in which the nucleus had been digested with DNase (17) ("native membranes"), and the latter preparation which was further treated at pH 11.5, resulting in total inactivation of the G and C functions (6, 18) (see Table 2). The amount of [^{3}H]isoproterenol locked in the receptor was proportional to the amount of membranes in the range 0.5–1.5 mg of protein. Depending on the batch, the native membranes locked 0.3–0.6 pmol of [^{3}H]isoproterenol/mg of protein and showed binding of 0.6–1.0 pmol of the specific β-adrenergic antagonist ^{125}I-labeled cyanopindolol/mg of protein.

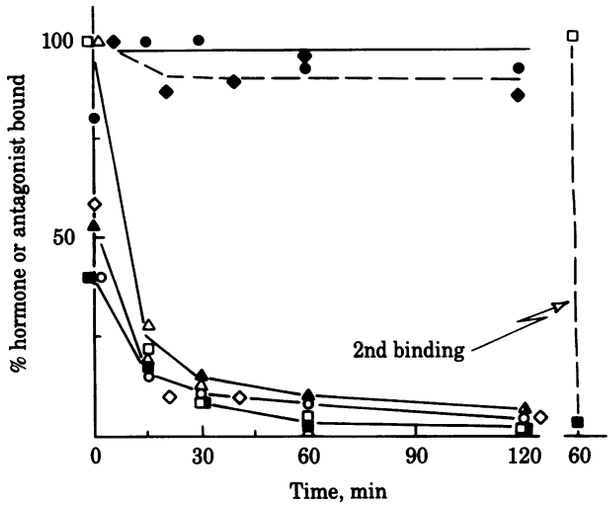

FIG. 1. Dissociation of agonists and antagonists from the β-adrenergic receptor in the presence and absence of DOC. The abscissa indicates the incubation time after centrifugation and resuspension in the presence of excess unlabeled ligand. The experiment was carried out as described in *Materials and Methods* with modifications as follows. After the initial incubation with radioactive ligand, the systems were divided into two equal parts; one part was further processed with reagents containing DOC at 1 mg/ml while the other was treated with the same reagents but without DOC (solid and open symbols, respectively). [^{3}*H*]*Isoproterenol systems*: The experiment was carried out with native membranes (◆ and ◇) (100% locking was 0.43 pmol/mg of protein) and also with pH 11.5 membranes (● and ○) (maximum locking, 1.4 pmol/mg). The latter preparation came from a different batch of native membranes. *Binding of the antagonist* [^{3}*H*]*alprenolol*: Native membranes, 1 mg/ml, were incubated with 10 nM [^{3}H]alprenolol in the absence and presence of 0.5 mM isoproterenol. The latter measured nonspecific binding. Systems were incubated for 10 min at 37°C to ensure effective binding of the antagonist (20) and then transferred to 4°C for 15 min. Incubation mixtures were diluted with an equal volume of incubation medium, with (■) or without (□) DOC at 2 mg/ml (final concentration, 1 mg/ml), centrifuged, and resuspended in the presence of isoproterenol exactly as described above for [^{3}H]isoproterenol systems. Immediately after resuspension, the 0 time sample in the absence of DOC (□) showed 0.7 pmol of [^{3}H]alprenolol bound specifically per mg of membrane protein and this value was defined as 100% [^{3}H]alprenolol bound. Nonspecific binding was 7%. To test whether DOC inactivated the receptor, a sample of 1 ml (0.5 mg of membrane protein) that had been incubated for 60 min in the presence of DOC was diluted and washed twice in incubation medium without DOC and then binding of fresh [^{3}H]alprenolol was tested on this sample in the absence of DOC. This second binding is indicated by an arrow on the right side of the figure pointing to the broken line. [^{3}*H*]*Propranolol systems*: Native membranes, 1 mg/ml, were incubated with 0.1 μM [^{3}H]propranolol in the absence and presence of 0.3 mM isoproterenol. Further processing was as described for [^{3}H]alprenolol. [^{3}H]Propranolol that remained bound after resuspension (0 time) was 0.5 pmol/mg of membrane protein and this value is defined as 100%. ▲ and △, with and without DOC, respectively.

Table 1 presents the raw data of a locking experiment. Nonspecific binding is only about 20% of total binding, and there is but a minor difference between nonspecific binding measured by addition of excess unlabeled isoproterenol and that measured by addition of the antagonist cyanopindolol. Therefore, there seems to be little doubt that the specific binding is to the β-adrenergic receptor and this is further supported below. Table 1 also shows that GTP and guanosine 5′-[γ-thio]triphosphate (GTP[S]), despite previous incubation at 37°C with

Table 1. Locking of [^{3}H]isoproterenol in the β-adrenergic receptor by DOC is unaffected by previous incubation with GTP or GTP[S]

Guanyl nucleotide added	Total cpm after locking*	Nonspecific cpm after locking: Excess isoproterenol	Nonspecific cpm after locking: Excess cyanopindolol
None	1,040 ± 20	220 ± 10	230 ± 10
GTP	990 ± 45	220 ± 5	225 ± 10
GTP[S]	920 ± 15	200 ± 5	215 ± 10

Systems in the standard medium were incubated for 10 min at 37°C with 0.6 μM [^{3}H]isoproterenol with or without 50 μM GTP or GTP[S]. After transfer to 4°C, total binding was measured on systems containing [^{3}H]isoproterenol as the only β-adrenergic ligand. Nonspecific binding was measured in the presence of 60 μM unlabeled isoproterenol and also on systems containing the antagonist cyanopindolol at 0.4 μM. The cpm bound represent 216 μg of membrane protein on the filters. Values are given as mean ± SEM for duplicate incubation systems, each analyzed in duplicate using samples of 108 and 216 μg of membrane protein (n = 8).

*[^{3}H]Isoproterenol specifically locked, calculated from the corrected dpm, was (pmol/mg of protein): none, 0.31; GTP, 0.31; GTP[S], 0.28. In a further experiment, membranes from a different batch were incubated with [^{3}H]isoproterenol with or without 0.1 mM GTP[S] for 30 min at 37°C. Subsequent locking at 2°C was (pmol/mg of protein) 0.45 and 0.46 with and without GTP[S], respectively.

the membranes and the labeled agonist, have no effect on the amount of isoproterenol locked subsequently in the cold by addition of DOC. Since these guanyl nucleotides apparently cause dissociation of the G component from the HR component (step *ii* above) (6, 10, 12, 21), it would seem that the G component is not involved in locking as carried out in the present study. Further evidence that the G component is indeed not required comes from experiments using membranes that had been treated at pH 11.5. The observations showing that pH 11.5 membranes no longer contain a functional G component are summarized in Table 2 (see also ref. 6). Yet, the β-adrenergic receptor in the alkali-treated membranes performed efficient locking of [^{3}H]-isoproterenol (see Figs. 1 and 4). Locking showed a typical saturation curve with respect to agonist concentration (Fig. 2). The same value as that shown in Fig. 2 for half-maximal locking was also obtained in another experiment, with a different batch of membranes, in which cyanopindolol served for measurement of nonspecific binding. The concentration of half-maximal locking (K_d in Fig. 2), calculated for (−)-[^{3}H]isoproterenol (13 nM) is almost identical with the K_d for binding of this agonist, determined at 1°C by other investigators (11 nM) by displacement of the labeled antagonist [^{125}I]iodohydroxybenzylpindolol (14). The virtual identity of the two numbers strongly supports the conclusion that locking of [^{3}H]isoproterenol occurs specifically in the β-adrenergic receptor. It furthermore suggests that locking freezes the steady state as it prevailed prior to addition of DOC. This is also supported by the finding that

Table 2. Summary of criteria for elimination of the G component and retention of the R component by treatment of native membranes at pH 11.5

Exp.	Native membranes	pH 11.5 membranes
1	Affinity of R for H is decreased by GTP (20, 21, 23)	Affinity of R for H is unaffected by GTP
2	H is locked in R by alkylation of SH in G	H is not locked in R by alkylating agent
3	—	R locked by alkylation of G in native membranes is unlocked by treatment at pH 11.5 (12)
4	After C inactivation by *N*-ethylmaleimide, G is measured by fusion with cyc$^-$ membranes (5, 24)	Fusion with cyc$^-$ membranes shows only 1% of G remaining, relative to native membranes
5	β-Adrenergic receptor is measured by [^{125}I]IHYP binding and by transfer to Fc adenylate cyclase (24)	β-Adrenergic receptor measured by binding as well as by fusion-transfer remains 80–100% of native membranes

H, hormone or synthetic agonist; C, catalytic unit of adenylate cyclase; cyc$^-$, S49 lymphoma cell variant that lacks a functional G component; Fc, a clone of Friend erythroleukemia cell line that has no β-adrenergic receptor; [^{125}I]IHYP, iodohydroxybenzylpindolol that had been used for binding assay of β-adrenergic receptor (25). Experiment 1: A batch of membranes was subjected to pH 11.5 treatment to eliminate the functional G component (18). Subsequently, those membranes, as well as native membranes, were treated to remove endogenous guanyl nucleotides (23). The apparent K_d at 37°C for (±)-norepinephrine was then calculated for both types of membranes from displacement curves of [^{125}I]IHYP (14). K_d values were as follows: native membranes without GTP, 1×10^{-6} M; with GTP, 6×10^{-6} M; pH 11.5 membranes without GTP, 2×10^{-6} M; with GTP, 2×10^{-6} M. In all three experiments with pH 11.5 membranes, GTP had no effect on the affinity of the β-adrenergic receptor for norepinephrine. Experiments 2 and 3: These experiments have been presented in detail elsewhere (12). Experiment 4: Native membranes were treated with *N*-ethylmaleimide in the cold, fused with cyc$^-$ membranes, and assayed for fluoride activation of adenylate cyclase as described (24). The pH 11.5 membranes were analyzed by the same procedure. Adenylate cyclase activity per fusion system (420 μg of membranes that had been treated with *N*-ethylmaleimide) was (pmol of cAMP/min) as follows: native membranes, 137; an equivalent amount of pH 11.5-treated membranes, 1.7. Experiment 5: Membranes were as in experiment 4. Native membranes (24 μg in 0.5 ml) specifically bound 9,500 cpm of [^{125}I]IHYP out of 150,000 cpm in the assay mixture. Nonspecific binding was 23%. The pH 11.5 membranes specifically bound 81% of the amount bound by native membranes. To measure the functional β-adrenergic receptor, membranes were treated with *N*-ethylmaleimide, fused with Fc cells, and assayed for isoproterenol-activated adenylate cyclase as described (18, 24). Adenylate cyclase activity per fusion system (140 μg of protein of native membranes) cAMP, pmol/min was as follows: basal, 4; isoproterenol-treated, 46; fluoride, 120. An equivalent amount of pH 11.5 membranes showed the following values: basal, 0.4; isoproterenol-treated 47; fluoride, 121. Controls of Fc cells or membranes treated at pH 12.5 and fused with Fc cells showed no activation by isoproterenol over the basal rate.

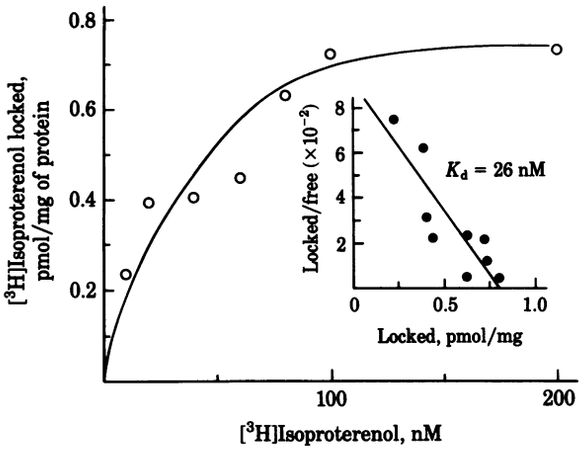

FIG. 2. Dependence of (±)-[^{3}H]isoproterenol locking on its concentration. The initial incubation time with different concentrations of agonist was 30 min.

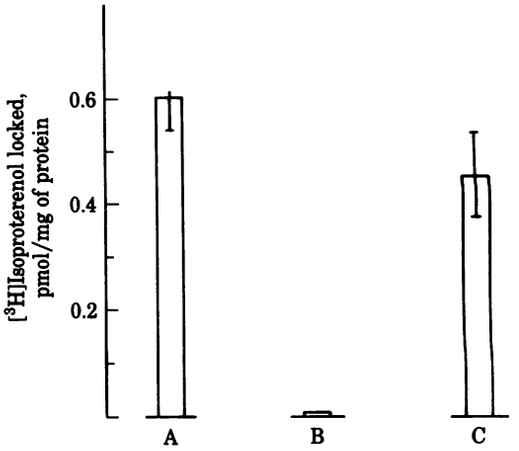

FIG. 3. Removal of DOC releases the locked [^{3}H]isoproterenol and permits relocking. In system A, locking was carried out as described in *Materials and Methods*. In systems B and C, the procedure was as in system A except that, after centrifugation, the pellet was resuspended in incubation medium containing DOC but lacking chaser isoproterenol. At the end of 30 min of incubation, the membranes in systems B and C were washed twice in incubation medium, with a 5-min incubation in the same medium between centrifugations to remove residual DOC. In system B, the membranes were finally suspended in incubation medium containing DOC at 1 mg/ml and 0.3 mM isoproterenol, incubated for 30 min at 4°C, and assayed. System B also served as a control for system C. After removal of DOC, system C was subjected to a second locking of [^{3}H]isoproterenol by the standard procedure.

locking decreases by 80% when hormone is added after DOC treatment (data not shown). As an additional check that the receptor site undergoing locking is the β-adrenergic receptor, a number of compounds were tested for their ability to compete with [^{3}H]isoproterenol. That this site is indeed the β-adrenergic receptor is shown in Table 3. (−)-Isoproterenol and (−)-epinephrine readily competed for the site while the (+)-enantiomers were inert at the concentrations tested. In another locking experiment, a concentration curve was run for (−)-epinephrine that indicated an affinity for the β-adrenergic receptor that was 1/10th of that of [^{3}H]isoproterenol (data not shown). Dopamine and phenylephrine were similarly without effect. Among the antagonists, the α-adrenergic blocker phentolamine was inactive while the β-adrenergic blocker propranolol inhibited locking of the labeled agonist. Also, the specific β-adrenergic receptor antagonist, cyanopindolol, has been shown to displace [^{3}H]isoproterenol (Table 1).

If the locking of hormone in the receptor is a conformational change that mimics the physiological reaction, it should be readily reversible when the detergent is removed. That this is so is shown in Fig. 3. After first locking the hormone in the receptor, the membranes were washed free of detergent. As a result, the receptor became unlocked and the hormone was released. Finally, readdition of detergent made the receptor receptive to relocking of hormone. Although Fig. 3 shows that the second locking was somewhat less effective, in many experiments it was as efficient as in the first cycle of locking (Fig. 4). Earlier studies indicated that the G component requires a divalent metal ion (10, 12, 26). In these investigations, there was no way to test whether the receptor, in the absence of a functional G component, would require a divalent metal ion in order to shift to a higher affinity conformation. The experiment shown in Fig. 4 indicates that Mg^{2+} is required by the receptor for efficient locking. Apparently, Mg^{2+} had no function in the initial binding of the hormone in the cold; this step could be carried out in the absence of Mg^{2+}, with or without EDTA, provided that the divalent metal ion was added subsequently, during the locking process (data not shown).

Table 3. Locking of [^{3}H]isoproterenol in turkey erythrocyte membranes fits the specificity of the β-adrenergic receptor

Ligand	Concentration, μM	(−)-[^{3}H]Isoproterenol,* μM	% inhibition of [^{3}H]isoproterenol locking
Catecholamine			
Dopamine	5.0	0.04	0
Phenylephrine	60.0	0.60	0
(+)-Isoproterenol	1.2	0.30	0
(+)-Isoproterenol	6.0	0.30	0
(−)-Isoproterenol	1.2	0.30	70
(−)-Isoproterenol	6.0	0.30	100
(−)-Epinephrine	0.5	0.04	50
(−)-Epinephrine	1.5	0.04	80
Antagonist			
α-Phentolamine	80.0	0.04	10
(±)-β-Propranolol	0.05	0.04	50
(±)-β-Propranolol	0.5	0.04	75

Experiments were carried out according to the standard locking procedure except as noted below. Antagonists, catecholamines, and phenylephrine were added to the membranes prior to addition of [^{3}H]isoproterenol.

* Supplied as the racemic (±) mixture. The concentration of (−)-[^{3}H]-isoproterenol is taken as 50% of the racemic mixture.

DISCUSSION

The present study shows that there is a qualitative difference between an agonist and an antagonist in their interaction with the receptor. Addition of DOC caused locking of the agonist while slightly increasing dissociation of the antagonist. The observation was all the more surprising because agonists are known to dissociate readily from the β-adrenergic receptor. Because of this rapid dissociation, measurement of direct binding of labeled β-adrenergic agonists to the receptor of turkey erythrocytes was hitherto not feasible. It is, however, important to emphasize that the locking phenomenon is of significance, not only because of the tight binding as such, but also because of the dramatic transition of the agonist-occupied receptor from an open to a closed conformation.

At present, it is not possible to prove that locking by detergent and locking in the HRG complex trapped by *N*-ethylmaleimide (12) are due to identical receptor conformations.

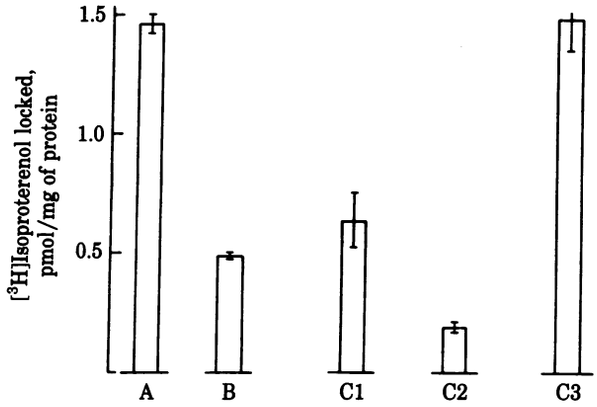

FIG. 4. Requirement for divalent metal ion in the locking process. Membranes that had lost their functional G component at pH 11.5 were used. Locking was carried out as described in *Materials and Methods* except as noted below. System A was treated according to the standard procedure. System B was treated according to the standard procedure except that 1 mM EDTA was used in place of Mg^{2+} from the stage of 1:10 dilution in the presence of DOC. System C1 was treated according to the standard procedure except that 1 mM EDTA was used in place of Mg^{2+} throughout the experiment. Systems C2 and C3 served to test the ability of the preparation treated with EDTA in the presence of DOC to relock [^{3}H]isoproterenol efficiently when Mg^{2+} was restored. These systems were treated as described for system C1 but the 30 min incubation was without isoproterenol. At the end of this incubation, DOC and EDTA were removed by washing the membranes twice with incubation medium with a 5-min incubation in the same medium in between centrifugations. The system C2 pellet was then suspended in 2 ml of incubation medium containing DOC at 1 mg/ml and 0.3 mM isoproterenol and incubated for 30 min prior to assay on the filters. It served as a control for the C3 system to give the amount of residual locked [^{3}H]isoproterenol prior to repeating the locking procedure. The C3 pellet was resuspended in 0.2 ml of incubation medium and the standard locking procedure was repeated.

However, considering that in both instances locking is highly specific for the agonist, it seems rather unlikely that there could exist two different conformations of the receptor at the hormone binding site, both with perfect fit to the agonist. It is therefore suggested that the induced fit at this site is the same, whether caused by interaction with the G component or by the detergent. The experiments conducted in the present study indicate that the G component is not required in the locking by DOC (Tables 1 and 2). It is, however, possible that a subunit of the G component that normally interacts with the receptor (26, 27) might persist in our preparation despite inactivation of the G component at pH 11.5 (Table 2). Even so, locking by DOC apparently represents the most elementary system for detecting an agonist-specific response of the receptor. It is noteworthy that the β-adrenergic receptor, if protected by isoproterenol, can be solubilized in DOC at 6 mg/ml while retaining its potential to activate an adenylate cyclase system (28). Current findings indicate that the agonist remains locked in the solubilized receptor (unpublished data). Further studies will determine whether locking by detergent is sufficient criterion to distinguish between a fully functional receptor and a nonfunctional receptor that retains specific binding of β-adrenergic ligands.

What can be learned about the conformational change in the receptor from the action of DOC? Since the detergent disrupts hydrophobic interactions, it may be assumed that the receptor is normally held in the open conformation by such interactions, either within the molecule itself or between the receptor and the membrane lipids. When these hydrophobic interactions are disrupted by the detergent, the full extent of induced fit between groups in the binding site and on the agonist is attained. Because Mg^{2+} is required, its interaction with carboxyl groups in the protein or with acidic lipids is probably also involved. On removal of the detergent, the original hydrophobic interactions of the receptor come into play again and the conformation of the binding site reverts to the open configuration. It seems reasonable to suggest that the hormone-occupied receptor, in its physiological interaction with the G component, proceeds through a similar sequence of conformational transformations as those produced by DOC. It is hoped that the present approach will yield further information about this early stage in the receptor–response pathway.

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