MOLECULAR PHARMACOLOGY, 20:224–226

SHORT COMMUNICATIONS

The Effect of Alprenolol on the *Beta*-Receptor and Adenylate Cyclase Activity in Rabbit Heart Membranes

V. A. TKACHUK AND M. WOLLEMANN

Department of Biochemistry, Moscow State University, Moscow, Union of Soviet Socialist Republics, and Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Received December 5, 1980; Accepted February 24, 1981

SUMMARY

TKACHUK, V. A., AND M. WOLLEMANN. The effect of alprenolol on the *beta*-receptor and adenylate cyclase activity in rabbit heart membranes. *Mol. Pharmacol.* **20**:224–226 (1981).

Incubation of rabbit heart membranes at 37° with 10^{-5} M alprenolol significantly increased the number of *beta*-receptor binding sites but did not change the adenylate cyclase activity. In the presence of guanine nucleotides, the effect of alprenolol incubation on the *beta*-receptor was enhanced. When the incubation was carried out at 4°, the alprenolol effect was lost.

In recent years, several studies have attempted to elucidate the molecular mechanism by which hormone agonists regulate the adenylate cyclase coupled to *beta*-receptors. It has been established that, when frog erythrocytes are incubated with *beta*-adrenergic agonists, a diminution of the maximal rate of isoproterenol-stimulated adenylate cyclase activity and a simultaneous decrease in the number of *beta*-receptors occurs (1) in the absence of guanine nucleotides. The addition of guanine nucleotides in high concentrations decreases the binding affinity of agonists, but not antagonists, to the *beta*-receptor (2, 3).

It has been demonstrated recently that turkey reticulocytes exposed to propranolol show an increase in the number of *beta*-receptors (4). On the other hand, a decrease of *beta*-receptors has been observed in adipocyte membranes after incubation with antagonists (5). Guanine nucleotides did not prevent the antagonist-induced decrease in the number of the receptors.

In addition, *beta*-adrenergic agonists are 7–70 times more active in competing for DHA[1] binding sites at 4° than at 37° in S49 lymphoma cells, whereas *beta*-adrenergic antagonists are less than 4-fold more potent at the lower temperature (6).

The purpose of the present work was to investigate the effects of incubation with the antagonist alprenolol on the catecholamine-stimulated adenylate cyclase activity and on the quantity of *beta*-adrenergic receptor binding sites in rabbit heart membranes.

(−)-Alprenolol was obtained from A. B. Hässle (Göteborg, Sweden), (±)-propranolol was obtained from Imperial Chemical Industries, Ltd. (Wilmslow, Cheshire, England), and all other fine chemicals were obtained from Sigma Chemical Company (St. Louis, Mo.). (−)-[^{3}H]Dihydroalprenolol (67 Ci/mmole) and [α-^{32}P]ATP (29 Ci/mmole) were obtained from New England Nuclear Corporation (Boston, Mass.).

Plasma membranes were prepared from New Zealand White rabbit ventricles as described elsewhere (7). Binding assay was performed in triplicate with aliquots of membranes containing 140 μg of protein in a volume of 500 μl containing 75 mM Tris-HCl buffer, pH 7.5, 25 mM $MgCl_2$, and 1–20 nM [^{3}H]DHA incubated at 37° for 10 min. Nonspecific binding was determined in the presence of 10 μM propranolol, according to the method of Lefkowitz *et al.* (8). Samples were filtered under vacuum on Whatman GF/C paper discs and washed four times with 5 ml of $MgCl_2$-containing buffer solution. The radioactivity of the dried filters was counted in a Nuclear Chicago Isocap liquid scintillation spectrometer.

The assay mixture for adenylate cyclase contained, in a final volume of 50 μl, 0.25 mM [α-^{32}P]ATP (10^6 cpm), 5 mM $MgCl_2$, 1 mM cyclic AMP, 25 mM Tris-HCl buffer (pH 7.5), 5 mM phosphoenolpyruvate, 25 μg of pyruvate kinase, and 87 μg of protein. Incubation was carried out at 37° for 20 min and was linear with time in the presence and absence of guanine nucleotides. Cyclic AMP was determined according to the method of White (9). All values represent the mean of three samples.

Plasma membranes were incubated with 10^{-5} M alprenolol at a given temperature for 10, 20, or 60 min. After incubation, the membranes were washed three or four

[1] The abbreviations used are: DHA, dihydroalprenolol; Gpp(NH)p, 5′-guanylylimidodiphosphate.

0026-895X/81/010224-03$2.00/0

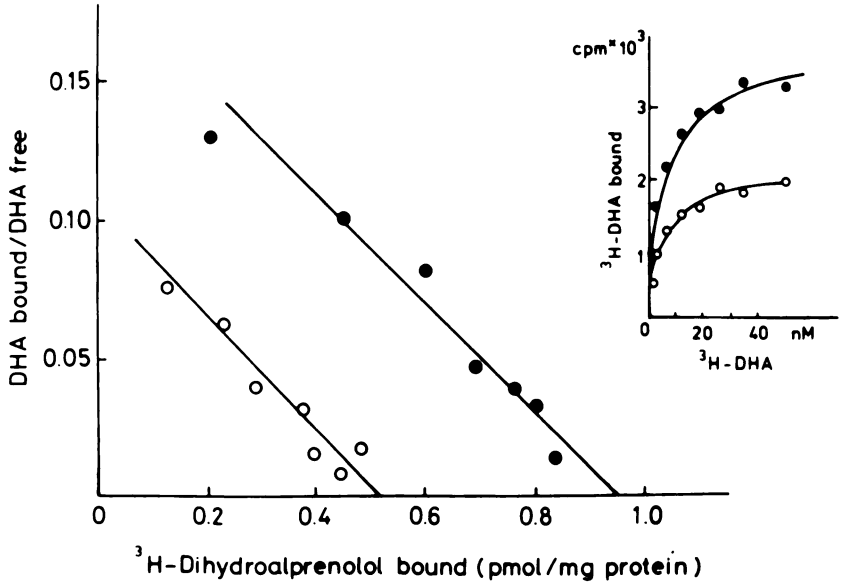

FIG. 1. *Scatchard plot of rabbit heart membrane preparation incubated with* (●) *or without* (○) 10^{-5} M *alprenolol at 33° for 20 min, washed four times with 25 m* M *Tris-HCl buffer (pH 7.5) containing 5 m* M $MgCl_2$, *and centrifuged at room temperature at 18,000 × g*

The *inset* shows the saturation curve of the control and alprenolol-treated membranes. The K_d for [^{3}H]DHA estimated from Scatchard and Lineweaver-Burk plots, both with and without incubation with alprenolol, was 5.7 nM.

times with 25 mM Tris buffer, pH 7.5, containing 5 mM $MgCl_2$, and centrifuged after each washing at 18,000 × *g* at room temperature. Protein determinations (10) were made after the incubation and washing.

Figure 1 and Table 1 show the effect of alprenolol incubation on the [^{3}H]DHA-binding and adenylate cyclase activity of rabbit heart membranes. The incubation enhanced the number of *beta*-receptors from 0.55 to 0.94 pmole/mg of protein, but did not change the adenylate cyclase activity significantly. During the incubation with buffer, there was no loss of [^{3}H]DHA binding sites: 0.48 ± 0.05 pmole of [^{3}H]DHA bound per milligram of protein before incubation, and 0.52 ± 0.04 after 20 min of incubation (mean of three experiments). Incubation with 10^{-5} M propranolol had the same effect as alprenolol (data not shown). Both drugs are classified as pure antagonists not having any agonist action (11).

Incubation of the membranes with a guanine nucleotide [either GTP or Gpp(NH)p] did not alter the number of *beta*-receptors, but incubation with alprenolol + guanine nucleotide enhanced the number of receptors even more than did alprenolol alone (Table 2).

Incubation with 10^{-5} M isoproterenol under the same conditions as with alprenolol decreased by 50% the specific binding of [^{3}H]DHA. When incubation with alprenolol, or with isoproterenol, was carried out at 4°, there was no significant effect of the incubation on the binding of [^{3}H]DHA (data not shown).

The results of our experiments and of those published

TABLE 1

Effect of incubation with alprenolol (alpr) on the activity of adenylate cyclase of rabbit heart membrane

Results are the mean ± standard error of the mean of three incubation samples in one representative experiment. Every experiment was performed at least three times in different membrane preparations. Incubations were carried out for 10 min at 37° and the preparations were washed three times at 20°. *Iso*, isoproterenol.

	Incubation with buffer	Incubation with 10^{-5} M alpr
	pmoles cyclic AMP/mg protein/min	
Basal	46.2 ± 2.1	45.0 ± 1.4
Iso, 10^{-4} M	55.2 ± 1.7	56.0 ± 2.0
Alpr, 10^{-5} M	45.3 ± 2.2	47.3 ± 2.8
Iso + alpr	32.0 ± 1.4	28.6 ± 1.2
Gpp(NH)p, 10^{-4} M	196 ± 8	186 ± 7
Gpp(NH)p + iso	292 ± 16	276 ± 8
Gpp(NH)p + alpr	201 ± 3	203 ± 10
Gpp(NH)p + iso + alpr	141 ± 6	137 ± 7

TABLE 2

Effect of alprenolol and guanine nucleotide incubation on DHA binding

Membranes were incubated in 1-ml solutions containing 24 mM $MgCl_2$-75 mM Tris-HCl (pH 7.5) for 20 min at 37° and were washed four times with 2 ml of the same buffer at 20°. For measurement of DHA-binding, 190–228 μg of protein were used. Incubation was carried out at 37° for 10 min in 0.5 ml of solution (25 mM $MgCl_2$-75 mM Tris-HCl (pH 7.5) with 10 nM [^{3}H]DHA ± 2 × 10^{-5} M propranolol. Each value shown is the mean ± standard error of the mean of three separate experiments.

Addition during incubation	[^{3}H]DHA bound
Buffer	0.465 ± 0.020
Alprenolol, 10^{-5} M	0.797 ± 0.086
GTP, 10^{-4} M	0.481 ± 0.045
GTP + alprenolol	1.107 ± 0.044
Gpp(NH)p, 10^{-4} M	0.445 ± 0.048
Gpp(NH)p + alprenolol	1.018 ± 0.109

in the literature (2-6) can be explained on the basis of transformation of *beta*-receptors in the presence of agonists or antagonists. Different phenomena are observed using antagonists or agonists during incubation: antagonists increase binding sites at 37°, but not at 4°. Guanine nucleotides increased the effect of antagonist during incubation (Table 2), but decreased the binding of agonists (12). These results are consistent with the data of Hanski and Levitzki (4) but at variance with the results published by Guidicelli and Agli (5).

It is somewhat surprising that a lower cyclase activity was exhibited by the heart membranes in the presence of isoproterenol + alprenolol than was exhibited in the presence of alprenolol alone, and that a lower cyclase activity was exhibited by the heart membranes in the presence of isoproterenol + alprenolol + Gpp(NH)p than was exhibited in the presence of alprenolol + Gpp(NH)p. One possible explanation for these differences is that isoproterenol at the high concentration used (10^{-4} M) has an inhibitory action on the cyclase as well as the stimulatory action mediated through the *beta*-receptor. Alprenolol, by selectively blocking the *beta*-receptor, would reveal the inhibitory action. Further research is required to provide evidence for this postulated inhibitory action of isoproterenol.

According to the model of Limbird *et al.* (13), there are differences in states of receptors. They are transformed under the action of antagonists and agonists. It can be explained that in the presence of agonist the receptor forms a complex with the guanyl nucleotide-binding protein and that in the presence of antagonists such a complex is not formed (13). Data given by the present work demonstrate that guanyl nucleotides influence not only the effects of agonists (12) but also the effects of antagonists; moreover, in the last case a very clear synergism is shown (Table 2).

The facts that the incubation of membranes with the antagonist increases the quantity of binding sites for [^{3}H]DHA (Fig. 1) and that guanyl nucleotides, which decrease the affinity of receptors for the agonists, potentiate the effect of the antagonist (Table 2) allow us to suppose that in the rabbit myocardial membranes some of the *beta*-adrenergic receptors are desensitized toward catecholamines.

In the present work, data are given concerning the increase in the quantity of *beta*-adrenergic receptors as the result of the incubation of membranes with alprenolol. This can be considered the resensitization of receptors for catecholamines *in vitro*. This process is temperature-dependent, it is increased in the presence of guanyl nucleotides (Table 2), and it does not affect the activity and regulatory properties of adenylate cyclase (Table 1).

REFERENCES

1. Mickey, J. Subsensitivity of adenylate cyclase and decreased β-adrenergic receptor binding after chronic exposure to (−)-isoproterenol *in vitro*. *J. Biol. Chem.* **250**:5727–5729 (1975).
2. Maguire, M. E., P. M. Van Arsdale, and A. G. Gilman. An agonist specific effect of guanine nucleotides on binding to the *beta*-adrenergic receptor. *Mol. Pharmacol.* **12**:335–339 (1976).
3. Lefkowitz, R. J., D. Mullikin, and M. G. Caron. Regulation of β-adrenergic receptors by guanyl-5′-yl imidophosphate and other purine nucleotides. *J. Biol. Chem.* **251**:4686–4692 (1976).
4. Hanski, E., and A. Levitzki. The absence of desensitization in the beta-adrenergic receptors of turkey reticulocytes and erythrocytes and its possible origin. *Life Sci.* **22**:53–60 (1978).
5. Guidicelli, Y., and B. Agli. Evidence for a second desensitized state of β-adrenergic receptor with low affinity for β-antagonists and normal reactivity towards β-agonists in adipocyte membranes previously exposed to β-antagonists. *Eur. J. Biochem.* **99**:457–462 (1979).
6. Insel, P. A., and M. Sanda. Temperature-dependent changes in binding to β-adrenergic receptors of intact S49 lymphoma cells. *J. Biol. Chem.* **254**: 6554–6559 (1979).
7. Tkachuk, A., P. V. Avdonin, and G. N. Baldenkov. Studying of the mechanism of heart adenylate cyclase activation by epinephrine and fluoride ion. *Biochimia Russ.* **42**:2005–2012 (1977).
8. Lefkowitz, R. J., D. Mullikin, and L. T. Williams. A desensitized state of the *beta*-adrenergic receptor not associated with high-affinity agonist occupancy. *Mol. Pharmacol.* **14**:376–380 (1980).
9. White, A. A. Separation and purification of cyclic nucleotides by alumina column chromatography. *Methods Enzymol.* **38**:41–46 (1974).
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
11. Kaumann, A. J., L. Birnbaumer. Characteristics of the adrenergic receptor coupled to myocardial adenylyl cyclase. Stereospecificity and determination of apparent affinity constants for β-blockers. *J. Biol. Chem.* **249**:7874–7885 (1974).
12. Limbird, L. E., D. M. Gill, and R. J. Lefkowitz. Agonist-promoted coupling of the β-adrenergic receptor with the guanine nucleotide regulatory protein of the adenylate cyclase system. *Proc. Natl. Acad. Sci. U. S. A.* **77**:775–779 (1980).
13. De Lean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).

Send reprint requests to: Dr. Maria Wollemann, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, 6701, Szeged POB 521, Hungary.