

Biphasic Adrenergic Modulation of β -Adrenergic Receptors in Man: *AGONIST-INDUCED EARLY INCREMENT AND LATE DECREMENT IN β -ADRENERGIC RECEPTOR NUMBER*

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Biphasic Adrenergic Modulation of β -Adrenergic Receptors in Man

AGONIST-INDUCED EARLY INCREMENT AND LATE DECREMENT IN β -ADRENERGIC RECEPTOR NUMBER

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ABSTRACT β -Adrenergic receptors in mononuclear leukocyte preparations were assessed with (-)[3 H]-dihydroalprenolol binding studies during the infusion of adrenergic agonists into normal human subjects. During the infusion of isoproterenol into seven subjects, mean (\pm SE) (-)[3 H]dihydroalprenolol binding increased from 25 ± 3 fmol/mg protein to 47 ± 8 fmol/mg protein ($P < 0.02$) at 0.5 h and 40 ± 3 fmol/mg protein ($P < 0.01$) at 1 h and decreased to 12 ± 1 fmol/mg protein ($P < 0.01$) at 4–6 h. During the infusion of epinephrine into three subjects, mean (-)[3 H]dihydroalprenolol binding increased from 32 ± 3 to 63 ± 3 fmol/mg protein ($P < 0.01$) at 0.5–1 h. By Scatchard plot analysis, these changes were attributable to changes in the number of available binding sites rather than changes in binding affinity. The observed changes in the number of (-)[3 H]dihydroalprenolol binding sites were not paralleled by changes in total mononuclear cell counts or in T lymphocyte, B lymphocyte, and monocyte distributions. Thus, we conclude that adrenergic agonists modulate the number of available β -adrenergic receptors on circulating mononuclear cells in a biphasic manner, with an early increment and a late decrement, in man. Further, the finding that the increase in pulse rate in response to a "pulse" infusion of isoproterenol was significantly greater after 0.5–1 h of agonist infusion suggests that the observed early agonist-induced increment in β -adrenergic receptor number on circulating cells is paralleled by increments in extra-vascular β -adrenergic receptor sensitivity.

INTRODUCTION

It has become increasingly apparent that a variety of hormones and neurotransmitters modulate their own

biologic expression through modification of the activity of their cellular receptors (1). The most commonly recognized pattern is that of a reciprocal relationship between ambient agonist concentration and receptor function as exemplified by insulin and its receptors; thus, high ambient insulin levels are associated with decreased receptor binding of insulin, whereas low ambient insulin levels are associated with increased receptor binding of insulin. Examples of a direct relationship between ambient agonist concentration and receptor function have been, however, recognized. For example, steroid hormones have been shown to induce their intracellular receptors (2) and prolactin has been reported to increase its plasma membrane receptor (3).

The effects of the catecholamines on their receptors have been extensively studied in animals. In general, measures that result in a relatively chronic decrease in catecholamine release result in increased adrenergic receptors and enhanced sensitivity to the biologic effects of the catecholamines (supersensitization), whereas measures that result in a relatively chronic increase in ambient catecholamine levels result in decreased adrenergic receptors and reduced sensitivity to the biologic effects of the catecholamines (desensitization) (4). As an example of the latter, Lefkowitz and co-workers (5, 6) have shown that exposure of frog erythrocytes to the β -adrenergic agonist isoproterenol in vitro, results in a decrease in β -adrenergic receptor number and in adenylate cyclase responsiveness and that similar changes follow the in vivo administration of isoproterenol to the frog. This phenomenon was reversible and was not a function of a change in receptor turnover. Similarly, isoproterenol administration to rats has been shown to result in a decrease in β -adrenergic receptor number in pineal plasma membranes (7). With the development of techniques for the assessment of β -adrenergic receptors on circulating leukocytes (8, 9),

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this observation has been extended to man in that ingestion of the β -adrenergic agonist terbutaline for as little as 3 d by normal subjects and patients with asthma was found to result in a reduction in the number of polymorphonuclear leukocyte β -adrenergic receptors (10). In contrast, intravenous infusion of the adrenergic agonists isoproterenol (11) and epinephrine (12) was not found to alter leukocyte β -adrenergic receptors. However, in the studies reported here we find a biphasic change in mononuclear cell β -adrenergic receptor number, with an early increase and a later decrease, during the intravenous infusion of the β -adrenergic agonist isoproterenol, and a similar early increase in mononuclear cell β -adrenergic receptor number during the infusion of the mixed adrenergic agonist epinephrine, in normal human subjects.

METHODS

Adrenergic agonist infusions. 13 normal young men consented to adrenergic agonist infusions performed, after an overnight fast, in the outpatient facilities of the Washington University Clinical Research Center. All subjects were free of recognizable cardiac disease, were normotensive, and had normal electrocardiograms. The electrocardiogram was continuously monitored throughout all agonist infusions. Agonists were diluted in saline containing ascorbic acid, 0.5 mg/ml, and infused for 1–6 h with a Harvard infusion pump (Harvard Apparatus Co., Inc., South Natick, Mass.). The agonists infused were isoproterenol (2.0 μ g/min, $n = 5$; 1.0 μ g/min, $n = 4$), epinephrine (5.0 μ g/min, $n = 3$), and norepinephrine (5.0 μ g/min, $n = 1$).

In four subjects infused with isoproterenol (1.0 μ g/min) and one subject infused with epinephrine (5.0 μ g/min), the increment in heart rate after a 10-min "pulse" infusion of isoproterenol (1.0 μ g/min) was measured 1 h before agonist infusion and again after 0.5–1 h of agonist infusion while the agonist infusion was continued. In three of these subjects, blood samples for binding studies were drawn before the isoproterenol pulses. Preliminary studies demonstrated that such isoproterenol pulses alone did not alter heart rate responses or binding results determined 1 h later.

Blood samples for the binding studies described below were drawn before and at various time points during agonist infusions with the exception of two 1.0- μ g/min isoproterenol infusions during which such samples were not drawn.

Mononuclear cell preparation. Mononuclear cells were separated from heparinized whole blood (80–100 ml) by the method of Böyum (13). The mononuclear cell fraction contains 80–85% lymphocytes, 10–20% monocytes, and <2% polymorphonuclear leukocytes with no more than one erythrocyte for each three to six mononuclear cells. The mononuclear cells were suspended in 50 mM Tris-HCl (pH 7.7) containing 10 mM MgCl₂ (incubation buffer) in a concentration of $\sim 10^8$ cells/ml and homogenized with 15 strokes of a Ten-Broek homogenizer (Fisher Scientific Co., St. Louis, Mo.) at 4°C to produce the mononuclear cell preparation used in the binding studies.

Binding studies. β -Adrenergic receptors in the mononuclear cell preparation were assessed with the labeled antagonist (–)[³H]dihydroalprenolol (51 Ci/mmol, New England Nuclear, Boston, Mass.) as described by Williams et al. (8). 300–400 μ g of mononuclear cell protein (14), derived from $\sim 6 \times 10^8$ cells, were incubated with 0.1–10.0 nM (–)[³H]dihydroalprenolol and incubation buffer containing 1 μ M

phentolamine (Ciba Pharmaceuticals Co., Div. Ciba-Geigy, Summit, N. J.) in a total incubation volume of 150 μ l for 20 min at 37°C. Incubations were terminated by dilution with 2 ml of cold (4°C) incubation buffer followed by rapid filtration through Whatman GF/C glass fiber filters (Whatman, Inc., Clifton, N. J.). The filters were then washed with 12 ml of cold buffer and, after appropriate preparation, counted in a liquid scintillation spectrometer. Specific binding of (–)[³H]dihydroalprenolol was considered to be that displaced by 1.0 μ M (–)propranolol and was $\sim 80\%$ of total binding under the conditions used. Phentolamine, in the concentration used (1.0 μ M), decreased nonspecific, but not specific binding. Specific binding increased linearly with increasing mononuclear leukocyte protein (85–500 μ g), the latter determined by the method of Lowry et al. (14).

Preliminary studies established that the mononuclear cell preparation contained binding sites characteristic of a β -adrenergic receptor. Binding of (–)[³H]dihydroalprenolol was saturable, rapid (with half-time of association and dissociation of <4 min), and of high affinity (K_d , 0.1–2.0 nM). The preparation exhibited the appropriate agonist potency sequence for displacement (isoproterenol > epinephrine > norepinephrine) and stereospecificity for displacement [(–)propranolol > (+)propranolol; (–)isoproterenol > (+)isoproterenol]. Scatchard plots were linear. The preparation was susceptible to desensitization (with a decrease in the number of binding sites but no change in binding affinity) during incubation with 1.0 μ M isoproterenol for 2 h in vitro.

Lymphocyte subpopulations. T lymphocytes and B lymphocytes were separated by the method described by MacDermott et al. (15) in samples obtained during three adrenergic agonist infusions (two with isoproterenol, one with epinephrine).

Statistics. Student's *t* tests for paired and unpaired data were used.

RESULTS

A biphasic change, an initial increase with a subsequent decrease, in (–)[³H]dihydroalprenolol binding to mononuclear cell preparations obtained before and during the infusion of isoproterenol for 4–6 h in seven normal human subjects is illustrated in Fig. 1. (–)[³H]dihydroalprenolol binding increased from a mean (\pm SE) of 25 ± 3 fmol/mg protein before infusion to 47 ± 8 fmol/mg protein ($P < 0.02$) at 0.5 h and 40 ± 3 fmol/mg protein ($P < 0.01$) at 1 h and decreased to 12 ± 1 fmol/mg protein ($P < 0.01$) at 4–6 h. Similar increments in (–)[³H]dihydroalprenolol binding, from 32 ± 3 to 63 ± 3 fmol/mg protein ($P < 0.01$), during the infusion of epinephrine over 1 h are also shown in Fig. 1. (–)[³H]dihydroalprenolol binding did not change (20 fmol/mg protein) during a single 1-h infusion of norepinephrine (not shown). By Scatchard plot analysis, these changes in (–)[³H]dihydroalprenolol binding were attributable to changes in the number, rather than the affinity, of binding sites as illustrated by the data from an isoproterenol infusion shown in Fig. 2 and the data from an epinephrine infusion shown in Fig. 3.

As shown in Table I, the observed changes in (–)[³H]dihydroalprenolol binding to mononuclear cell preparations during adrenergic agonist infusions were not paralleled by changes in the proportion of T

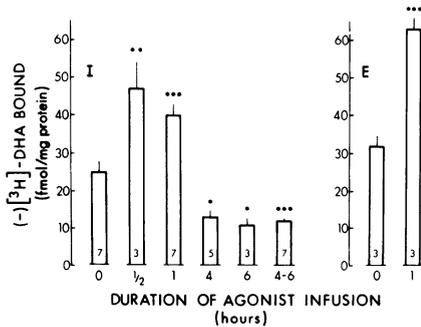


FIGURE 1 Mean (\pm SE) specific (-)[3 H]dihydroalprenolol [(-)[3 H]-DHA] binding to mononuclear cell preparations, obtained before and at the indicated times, during the infusion of isoproterenol (I, left) in seven subjects and the infusion of epinephrine (E, right) in three subjects. The numbers at the base of each column indicate the number of observations at the corresponding duration of agonist infusion. (*) $P < 0.05$, (**) $P < 0.02$, and (***) $P < 0.01$.

lymphocytes, B lymphocytes, or monocytes. Total leukocyte and mononuclear cell counts increased during epinephrine but not isoproterenol infusions.

In five subjects pulsed with isoproterenol for 10 min before and after 0.5 or 1 h of isoproterenol ($n = 4$) or epinephrine infusions, the increment in pulse rate rose from 9 ± 2 beats/min before agonist infusion to 17 ± 4 beats/min ($P < 0.05$) during agonist infusion. Samples for binding studies were obtained in three of these subjects. As shown in Fig. 4, in increments (-)[3 H]dihydroalprenolol binding to mononuclear preparations were parallel by increments in the heart rate response to pulses of isoproterenol.

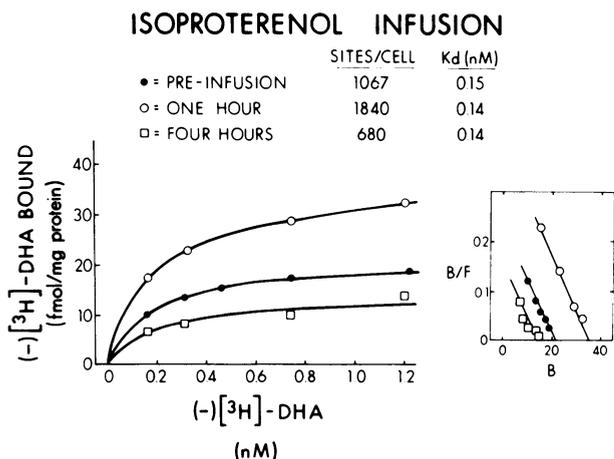


FIGURE 2 Specific (-)[3 H]dihydroalprenolol [(-)[3 H]-DHA] binding to mononuclear cell preparations obtained before and after one and four hours of isoproterenol infusion ($2.0 \mu\text{g}/\text{min}$) in a single subject. The inset shows Scatchard plots of the data. B, (-)[3 H]dihydroalprenolol bound (fmol/mg protein). B/F, ratio of bound to free (-)[3 H]dihydroalprenolol.

EPINEPHRINE INFUSION

| | SITES/CELL | Kd (nM) |
|------------------|------------|---------|
| ● = PRE-INFUSION | 810 | 0.20 |
| ○ = ONE HOUR | 1710 | 0.19 |
| □ = FOUR HOURS | 870 | 0.20 |

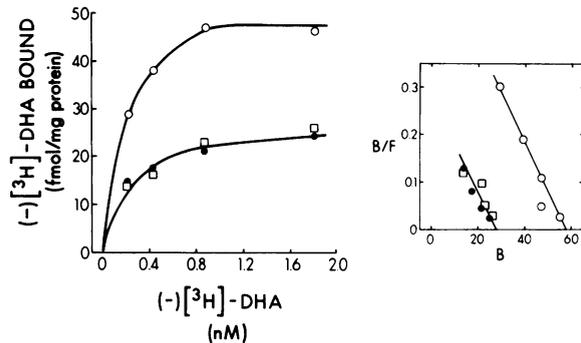


FIGURE 3 Specific (-)[3 H]dihydroalprenolol [(-)[3 H]-DHA] binding to mononuclear cell preparations obtained before and after 1 and 4 h of epinephrine infusion ($5.0 \mu\text{g}/\text{min}$) in a single subject. The inset shows Scatchard plots of the data. B, (-)[3 H]dihydroalprenolol bound (fmol/mg protein). B/F, ratio of bound to free (-)[3 H]dihydroalprenolol.

DISCUSSION

These data demonstrate an early 88 and 98% increase in mean (-)[3 H]dihydroalprenolol binding to circulating mononuclear leukocyte preparations obtained during the intravenous infusion of the β -adrenergic agonist isoproterenol and of the α - and β -adrenergic agonist epinephrine respectively into normal human subjects. This occurred within 30 min of the start of the infusions and was transient. With continued isoproterenol infusion (-)[3 H]dihydroalprenolol binding decreased to 48% of preinfusion values after 4–6 h. These changes in binding were attributable to sequential increases and decreases in the number, rather than the affinity, of available (-)[3 H]dihydroalprenolol binding sites and, therefore, sequential increases and decreases in the number of available mononuclear cell β -adrenergic receptors during adrenergic agonist infusions. Because the distribution of T lymphocytes, B lymphocytes, and monocytes did not change in parallel with the changes in β -adrenergic receptor number during agonist

TABLE I
Distribution of Mononuclear Cell Types during the Infusion of Adrenergic Agonists (Percent)

| | Duration of agonist infusion, h | | | |
|---------------|---------------------------------|------------|------------|------------|
| | 0 | 1 | 2 | 4 |
| T lymphocytes | 65 ± 4 | 58 ± 5 | 58 ± 6 | 50 ± 5 |
| B lymphocytes | 23 ± 1 | 21 ± 3 | 22 ± 2 | 27 ± 3 |
| Monocytes | 6 ± 1 | 8 ± 4 | 9 ± 5 | 8 ± 3 |

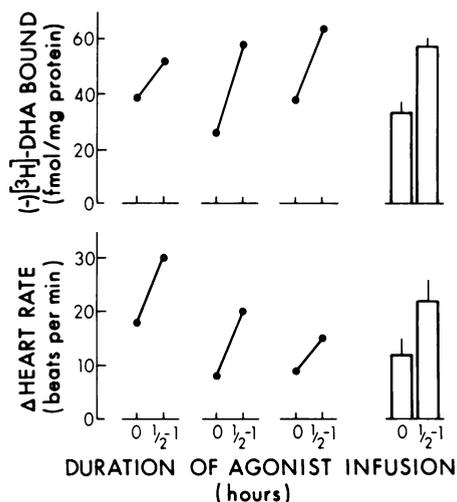


FIGURE 4 Increments (Δ) in heart rate (lower panel) in response to a pulse of isoproterenol before and after 0.5–1 h of isoproterenol (1.0 $\mu\text{g}/\text{min}$, $n = 2$) or epinephrine (5.0 $\mu\text{g}/\text{min}$) infusions along with specific (–)[^3H]dihydroalprenolol [(–)[^3H]-DHA] binding to mononuclear preparations drawn just before the isoproterenol pulses (upper panel). Mean (\pm SE) values are shown at right.

infusions and the total mononuclear cell count did not change during isoproterenol infusions, the observed sequential increments and decrements are best attributed to increments and decrements in the number of available β -adrenergic receptors per circulating mononuclear cell, rather than the flux of subpopulations of mononuclear cells that may have relatively high numbers of β -adrenergic receptors (16, 17) into, and out of, the circulation. Although it is conceivable that changes in the number of other mononuclear cell classes, which we did not characterize, may have occurred, these data do not categorically exclude that possibility.

To our knowledge, an agonist-induced increase in β -adrenergic receptors has not been observed in previous *in vivo* studies. Keabian et al. (7) observed a decrease in pineal β -adrenergic receptors as early as 30 min after the injection of isoproterenol into rats and Mukherjee et al. (6) noted a small decrease in erythrocyte β -adrenergic receptors as early as 1 h after the injection of isoproterenol into frogs. In human subjects, Tuck and Krall (11) found no significant change in the number of β -adrenergic receptors in mononuclear cells during the infusion of isoproterenol. One consistent difference between these studies and this study is the use of considerably larger doses of isoproterenol in the former studies. Shamoon et al. (12) observed no change in mononuclear cell β -adrenergic receptors during 4-h infusions of epinephrine, in doses comparable with those we used, into humans although the time points examined by these investigators are not clear.

Similarly, an agonist-induced increase in β -adrenergic receptors has not been observed in the vast majority of *in vitro* studies. There are, however, some possible precedents for an agonist-induced increase in adrenergic receptor activity in *in vitro* studies. Ruffolo et al. (18) have reported that low concentrations of the α -adrenergic agonists methoxamine (1.0 μM) and phenylephrine (1.0 μM), and to a lesser extent norepinephrine (0.1 μM), resulted in increased binding of [^3H]dihydroazapetine, a putative α -adrenergic antagonist, to binding sites in rat *vas deferens* membranes. Recently, Auerbach and Klein (19), studying β -adrenergic receptors on cultured intact rat pinealocytes, noted that during incubation with norepinephrine and epinephrine there was a rapid 200-fold increase in the amount of agonist required to displace 50% of the specifically bound ligand.

Recent observations reported from Hirata et al. (20, 21) provide a plausible mechanism for the observed agonist-induced increase in β -adrenergic receptor number. These investigators demonstrated that isoproterenol applied to the external surface of rat reticulocyte ghosts rapidly stimulates the synthesis of phosphatidylcholine (20) and that S-adenosyl-L-methionine stimulated phosphatidylcholine synthesis resulted in a linear increase in the number of available β -adrenergic receptors over 60 min (21). They emphasized that this increase in β -adrenergic receptor number is dependent upon the integrity of the ghost plasma membranes and reasoned that the increase in available β -adrenergic receptors was caused by the unmasking of cryptic receptors.

Although it is reasonable to expect that changes in β -adrenergic receptor number on circulating mononuclear cells are paralleled by changes in β -adrenergic receptor number on extra-vascular target cells and by changes in cellular sensitivity to the biologic actions of agonists that interact with β -adrenergic receptors, these premises cannot be assumed. Support for these premises in this study is provided by our observation that the heart rate responses to a pulse infusion of isoproterenol were significantly increased after 0.5–1 h of agonist infusion in five subjects. Further, this increase in a response mediated through myocardial β -adrenergic receptors was paralleled by a measured increase in the number of mononuclear cell β -adrenergic receptors in the three subjects in whom binding studies were also performed.

The *in vivo* observation of an early agonist-induced increase in β -adrenergic receptor number has potential physiologic implications with respect to the modulation of sympathoadrenal activity. The early agonist-induced enhancement of postsynaptic β -adrenergic receptor sensitivity would be expected to augment β -mediated biologic responses to released catecholamines. Furthermore, in view of the finding that cate-

cholamines are cleared through β -adrenergic mechanisms (22), agonist-induced enhancement of β -adrenergic receptor sensitivity may well explain the recent observation that the catecholamines accelerate their own metabolic clearance (23).

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