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Research Article

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In Vivo Regulation of β-Adrenergic Receptors on Mononuclear Leukocytes and Heart

Assessment of Receptor Compartmentation after Agonist Infusion and Acute Aortic Constriction in Guinea Pigs

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Abstract

In animals injected with a bolus of isoproterenol, β-adrenergic receptors in both mononuclear leukocytes (MNL) and heart were sequestered away from the cell surface, and the time course (0–120 min) and dose-response patterns were similar in the two tissues. In guinea pigs given a constant infusion of isoproterenol, 0.15 mg/(kg · h), down-regulation of total receptor number occurred more quickly and to a greater extent in the MNL than in the heart.

We also compared receptor sequestration after aortic constriction-induced acute heart failure. Negligible sequestration (9%) of β-adrenergic receptors occurred in the MNL of animals treated in this manner, whereas the number of receptors in the sarcolemmal fraction decreased 61%. This selective sequestration of cardiac receptors may result from the action of high concentrations of norepinephrine (which is selective for β_1 over β_2 receptors) present at sympathetic nerve-cardiac cell synapses.

We conclude that although receptor redistribution occurs similarly in MNL and heart in response to a circulating nonselective agonist, β-adrenergic receptor redistribution may occur selectively in the heart in response to such stimuli as aortic constriction-induced acute heart failure that activate the sympathetic nervous system.

Introduction

Stimulation of β-adrenergic receptors by agonists activates adenylate cyclase, but with continued stimulation receptor-mediated responses become blunted or desensitized. This agonist-promoted desensitization has been observed with a variety of cell types. Several different molecular mechanisms of desensitizations have been proposed (1, 2). One of these is the rapid (less than a few minutes) redistribution or internalization of β-receptors into membrane vesicles where the receptors are uncoupled from the stimulatory guanine nucleotide-binding protein (G_s)[1] and from the catalytic unit of adenylate cyclase. Two methods have been used to discriminate between receptors on the cell surface and those in intracellular vesicles. One is to fractionate cell homogenates by differential centrifugation; receptors present in very light membrane fractions are presumed to represent intracellular receptors (1, 3, 4). The second method is to use intact cells and to compare the binding of a hydrophobic antagonist with that of the hydrophilic β-adrenergic antagonist CGP-12177, which binds to cell surface receptors but cannot penetrate the cell membrane to detect intracellular receptors (5–7). Although redistribution of receptors by agonists occurs in a few minutes, down-regulation (of receptor number) occurs with a time course of hours or days. These two events, β-adrenergic receptor redistribution and β-adrenergic receptor down-regulation, may contribute to the pathophysiology of various disease states (8, 9).

Mononuclear leukocytes (MNL) are easily accessible cells for investigating the regulation of human β-adrenergic receptors (5, 8, 10). We have found that β agonists added to human MNL in vitro can promote redistribution, and that this redistribution may contribute to desensitization of receptor function (5, 6). Despite evidence of receptor redistribution from in vitro studies, negligible redistribution of human MNL β-receptors occurs during vigorous exercise, a 30-min of infusion of isoproterenol, or a 6-d treatment with the β-adrenergic agonist terbutaline (11, 12).

The purpose of this study was to explore further the utility of MNL as a mode for receptors on solid tissues. In particular, we have determined whether changes in the number and location of β-receptors on MNL accurately reflect changes in β-receptors that occur in the heart.

Methods

Infusion of isoproterenol and induction of heart failure. For short time-course experiments (< 120 min) male Hartley guinea pigs (400–500 g, 4–6 wk, Simonsen Co.) received either (−)-isoproterenol (0.05–0.2 mg/kg in 10 mM HCl) or 10 mM HCl by subcutaneous injection. For longer time periods the animals were anesthetized with ketamine (110 mg/kg i.m.) and Alzet model 2001 or 2002 osmotic minipumps (Alza Corp., Palo Alto, CA) were then implanted subcutaneously. These minipumps were used to deliver isoproterenol or vehicle (HCl 10 mM) at a rate of 0.15 mg/(kg · h) for up to 21 d. At the desired time of sacrifice, animals were anesthetized with ketamine (80 mg/kg i.m.) and xylazine (8 mg/kg i.m.), and blood was obtained via direct left ventricular puncture and mixed with EDTA (5 mM final) and placed on ice. Plasma was frozen for subsequent determination of isoproterenol and norepinephrine levels, which were measured radioenzymatically (13). The heart was removed and perfused with ice-cold 50 mM Tris-HCl (pH 7.4), and the left ventricle was trimmed and frozen at −70°C.

To establish an acute pressure overload on the heart, animals were anesthetized with ketamine/xylazine, intubated, and ventilated with supplemental oxygen on a Harvard respirator (Harvard Apparatus Co., Inc., S. Natick, MA). A left thoracotomy was performed and a metal

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1. *Abbreviations used in this paper:* G_s, stimulatory guanine nucleotide-binding protein; ICYP, iodocyanopindolol; IPIN, iodopindolol; MNL, mononuclear leukocyte(s).

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clip was placed around the ascending aorta causing an 80–90% constriction for 1 h. This usually led to rapid cardiac dilatation, sympathetic stimulation, elevated norepinephrine levels, and hypotension. 6 of 36 animals did not survive for 1 h (range of survival, 5–20 min) and these were excluded from analysis. At the time of sacrifice (1 h) left ventricular pressure was obtained via a 22-gauge needle attached to a pressure transducer, blood was obtained, and the heart was removed as described above. A control group of animals underwent thoracotomy without subsequent aortic constriction.

Lymphocyte preparation and radioligand binding. Techniques for preparation of guinea pig lymphocytes and radioligand binding to β-receptors involved modifications of previous studies with human cells (5, 6, 14). At the time of sacrifice of animals, blood was immediately placed into tubes containing EDTA (5 mM final) and was transferred into tubes containing an equal volume ice-cold PBS. All steps thereafter were performed at 4°C. The blood was centrifuged at 500 *g* and the platelet rich plasma was removed. An equal volume of PBS was added, the tube was centrifuged at 500 *g* again, and the supernatant was discarded. PBS was added to bring the volume to twice that of the starting blood volume. 10 ml of Ficoll-Hypaque was subfused slowly and samples were then centrifuged at 1400 rpm for 15 min. MNL were collected from the interface and washed three times in PBS and then resuspended in Dulbecco's modified Eagle's medium with 20 mM Hepes (DMEH, pH 7.4) and 1 mg/ml bovine serum albumin. This method involves longer and faster centrifugations than those typically used for preparation of human MNL (5, 14); this was necessary to prepare MNL free of platelets and red cells. Over 90% of cells were able to exclude trypan blue. Radioligand binding were conducted in a total volume of 0.5 ml DMEH containing 2–3 × 10^5 MNL, varying concentrations of −[^{125}I]iodopindolol (IPIN) between 10 pM and 120 pM and either buffer, 1 μM (−)-propranolol (Ayerst Laboratories New York, NY), or 1 μM CGP 12177 (kindly provided by Dr. M. Staehelin, Ciba Geigy, Basel, Switzerland). Incubations were continued at 4°C for 40 h, at which time the cells were filtered over Whatman GF/C filters (Whatman Inc., Clifton, NJ) and washed in 20 ml of PBS at room temperature. This lengthy incubation was necessary to achieve steady state in binding to sequestered receptors at 4°C (5). The difference between total binding and binding in the presence of propranolol represents the total number of receptors; the difference between total binding and binding in the presence of CGP-12177 represents surface receptors (5, 6). The number of sequestered or redistributed receptors is defined as the total number of receptors minus the number of surface receptors. Studies with β_1 and β_2 selective agents indicate that guinea pig MNL possesses a pure population of β_2-adrenergic receptors, as do human MNL (5 and data not shown).

cAMP accumulation in MNL. Incubations were begun by adding 0.1 ml of ice-cold cells (2–5 × 10^5) to 0.9 ml of DMEH at 37°C containing, 1 mg/ml BSA, 1 mM isobutylmethylxanthine, and 0.1 mM Ro-1724 (kindly provided by Dr. H. Sheppard, Roche Laboratories, Nutley, NJ) to inhibit phosphodiesterase activity, 10 μg/ml superoxide dismutase and 10 μg/ml catalase (to prevent oxidation of isoproterenol [15]) in the absence or presence of 10 μM isoproterenol. The incubation was stopped after 2 min by centrifugation at 10,000 *g*. The supernatant was aspirated and the pellet was resuspended in 100 μl 50 mM sodium acetate (pH 4.0) and 0.2 mM isobutylmelthyxanthine. The tubes were placed in a boiling water bath for 5 min, and then were frozen. Aliquots were assayed for cAMP using a competitive binding protein method as previously described (5, 11).

Preparation of sarcolemmal and light vesicle membranes and assay of β-adrenergic receptors in cardiac membranes. We isolated two fractions from excised left ventricles; a purified sarcolemmal fraction in which β-adrenergic receptors are responsive to agonist, and a lighter membrane fraction of receptors that are unresponsive to agonist. Details of this method have been described previously (3, 16). Briefly, after extraction of contractile proteins with 750 mM NaCl, the samples were rigorously homogenized. A "crude" fraction was isolated by removal of the supernatant of a 500-*g* centrifugation. The light vesicle fraction was obtained by centrifuging the supernatant of a 45,000-*g* centrifugation at 137,000 *g* for 90 min. We have previously shown that receptors in this light vesicle fraction are functionally uncoupled from G_s (the stimulatory guanine nucleotide binding protein) and that this fraction has little adenylate cyclase, 5′-nucleotidase, or Na^+-K^+ ATPase activity and is not enriched in markers for lysosomes or mitochondria (17). Sarcolemmal membranes were obtained by serial washings of the pellet of the 45,000-*g* centrifugation and then taking the supernatant of a 17,000-*g* centrifugation and centrifuging at 137,000 *g*. The resulting rim above the pellet was considered the sarcolemma fraction and is enriched in adenylate cyclase and Na^+-K^+-ATPase activity; binding of agonist to β-adrenergic receptors in this fraction is regulated by guanine nucleotides (17–19). Protein was determined by the method of Lowry using bovine serum albumin as the protein standard (20).

Binding of [^{125}I]iodocyanopindolol (ICYP) to β-adrenergic receptors was measured by incubating membranes in triplicate with varying concentrations of radioligand (50–600 pM) at 25°C for 1 h. Binding was terminated by diluting samples to 10 ml with ice-cold buffer, filtering over Whatman GF/B filters that had been presoaked in 2% polyethyleneimine and washing the filters with 10 ml of ice-cold buffer. We used filters soaked in polyethyleneimene to minimize loss of receptors that might be present in small membrane fragments and that might therefore not be trapped in untreated glass fiber filters (21). Radioactivity retained on filters was determined using a gamma counter at 86% efficiency. Nonspecific binding for both sarcolemmal and light vesicle fractions was determined by [^{125}I]ICYP binding in the presence of 1 μM(−)-propranolol and was routinely 10–20% of total binding for both membrane fractions.

Adenylate cyclase assay. Adenylate cyclase was assayed by the method of Salomon et al. (22) in a buffer containing 50 mM Tris-HCl, 10 mM Mg Cl_2, 0.3 mM cAMP, 1 mM ATP, 100 μM GTP, 20 mM creatine phosphate, 50 U/ml creatine phosphokinase, 800,000 cpm [^{32}P]ATP, and in some tubes 100 μM isoproterenol, 10 μM forskolin, or 10 μM guanylylimidodiphosphate [Gpp(NH)p]. Incubations lasted 20 min at 30°C and cAMP was isolated by sequential chromatography, using Dowex (Dow Corning Corp., Midland, MI) and alumina.

Data analysis. Radioligand binding data were analyzed using a computer program that performs iterative nonlinear regression to fit the specific binding to an equation describe binding of ligands to a single class of homogeneous binding sites. The experiments were all performed with triplicate data points and were conducted at least twice with similar results. Unless stated otherwise, the data are presented as mean±SE of the mean. To determine statistical significance of we calculated unpaired two-tailed *t* tests.

Results

In vitro redistribution of β-adrenergic receptors on MNL by isoproterenol. We have previously demonstrated that incubating human blood with 10 μM isoproterenol for 10 min redistributed up to 50% of the β-adrenergic receptors on MNL without changing the total receptor number (5, 11). Guinea pig MNL contained 912±83 β-adrenergic receptor sites per cell with a K_d for [^{125}I]IPIN of 0.03±0.004 nM (n = 15). Of these 90.2%±0.7% of the receptors were on the cell surface, as defined by the ability of CGP-12177 to inhibit [^{125}I]IPIN binding. Thus, only 10% of β-receptors were sequestered on MNL under basal conditions. The dose-response pattern for redistribution of β-receptors produced by incubation of guinea pig blood with isoproterenol in vitro for 10 min is shown in Fig. 1. The EC_{50} concentration of isoproterenol was 20 nM. About 50% of the [^{125}I]IPIN sites were maximally redistributed by isoproterenol (1 μM) in this short-term incubation. These results with guinea pig cells are similar to those previously observed for human cells (5).

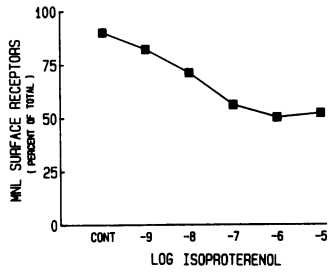


Figure 1. Dose-response pattern for redistribution of β-adrenergic receptors produced by incubating guinea pig blood with isoproterenol for 10 min. About 50% of the [^{125}I]IPIN sites were maximally redistributed by isoproterenol. This figure is a single experiment performed with triplicate determinations. A repeat experiment gave similar results.

Isoproterenol-induced redistribution of β-adrenergic receptors on MNL and heart in vivo. Fig. 2, *A* and *B*, shows results of experiments that examine redistribution of β-receptors on circulating MNL and in heart membranes after subcutaneous injection of guinea pigs with 0.15 mg/kg of isoproterenol. Each time point in Fig. 2 *A* represents the pooled blood of two to three animals. Loss of receptors from the cell surface occurred within the first 15 min. Between 15 and 90 min the percentage of sequestered receptors remained relatively constant at about 25% of the total receptors. The total number of receptors did not change in the first 90 min but receptor number began to "down-regulate" thereafter. As shown in Fig. 2 *B* the number of cardiac sarcolemmal receptors decreased by about 25%

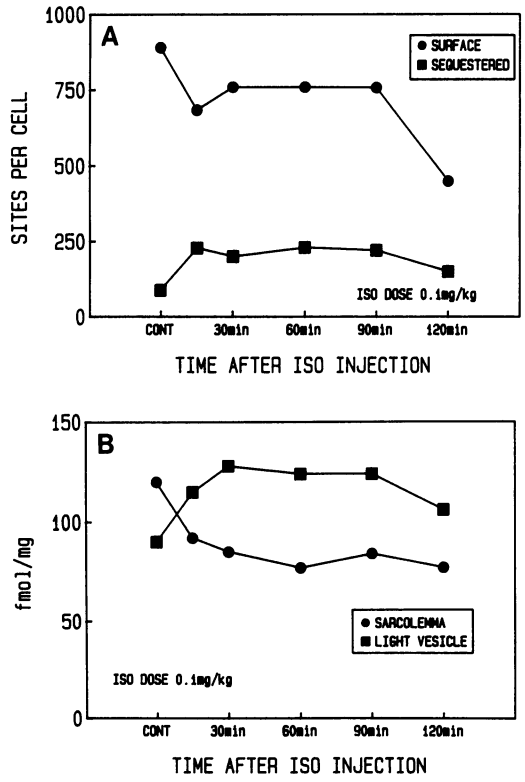


Figure 2. The time course of redistribution of β-adrenergic receptor on MNL and heart membranes. Animals were given 0.15 mg/kg isoproterenol subcutaneously. Each time point in *A* represents the pooled blood of three animals; each time point in *B* represents the pooled ventricles of three animals. The data points represent the B_{max} determined from saturation isotherms using four to five concentrations of radioligand performed in triplicate. The experiment was repeated twice with similar results.

within 15 min after isoproterenol administration and this agonist-induced decrease was maximal by 30 min. This decrease in sarcolemmal receptors appeared to represent receptor internalization (i.e., sequestration), as the number of receptors that were detectable in the light vesicle fraction increased over a similar time course. Thus, the kinetics of sequestration of β-adrenergic receptors in response to a single injection of isoproterenol is similar in MNL and heart.

Fig. 3 shows the pooled results of six experiments with two or three animals per experiment, each killed 1 h after isoproterenol injection (0.15 mg/kg). The number of β-adrenergic receptors on the surface of MNL decreased from 829±73 sites per cell in control to 654±80 sites per cell (a 21% decrease in the number of surface sites). In the heart, the number of sarcolemmal β-receptors decreased from 106±8 to 71±6 fmol/mg, representing a decrease of 33%. Thus the extent of sequestration (defined as percent of control number of surface sites) after an injection of guinea pigs with 0.15 mg/kg isoproterenol is roughly similar in heart and MNL.

As shown in Fig. 4, 30 min after injection of isoproterenol, isoproterenol-stimulated cAMP accumulation in MNL and adenylate cyclase activity in sarcolemma were substantially blunted (desensitized). In contrast, the responsiveness of sarcolemmal adenylate cyclase to 10 μM forskolin and 10 μM Gpp(NH)p did not change after isoproterenol injection (forskolin: 455 → 420 pmol min^{-1} mg^{-1}, Gpp(NH)p: 178 → 179 pmol min^{-1} mg^{-1}, n = 2). Thus, desensitization of cardiac and MNL β-adrenergic receptor mediated cAMP accumulation was observed at a time of substantial sequestration but only minimal down regulation of β-adrenergic receptors. It should be noted, however, that the extent of desensitization exceeded the loss of surface receptors in both tissues.

Fig. 5 shows results comparing the loss of surface β-adrenergic receptors in MNL and heart 1 h after injection of four different doses of isoproterenol. The measured isoproterenol levels ranged from 82 pg/ml (0.4 nM) when the dose was 0.05 mg/kg, to 12,814 pg/ml (60 nM), at a dose of 0.20 mg/kg. Thus, at several different doses and blood levels of isoproterenol, the extent of sequestration appears to be similar in MNL and heart.

Isoproterenol-induced down-regulation of β-adrenergic receptors on MNL and heart in vivo. Fig. 6 *A* shows the kinetics of down-regulation of β-adrenergic receptors on guinea pig

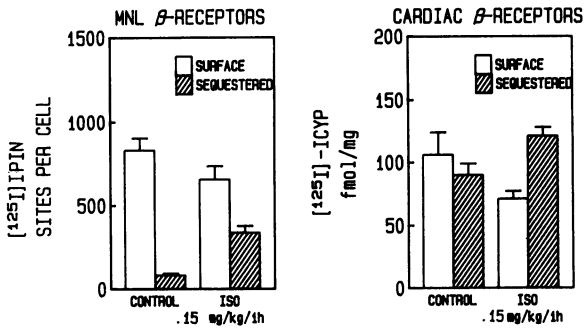


Figure 3. The effects of isoproterenol injection on redistribution of β-adrenergic receptors in MNL and heart. Animals were injected with 0.15 mg/kg isoproterenol subcutaneously, and killed 1 h later. Data are expressed as [^{125}I]IPIN binding sites/cell for MNL and [^{125}I]ICYP (fmol/mg) for heart, and are the mean±SEM of six experiments using the pooled tissue of three animals per experiment.

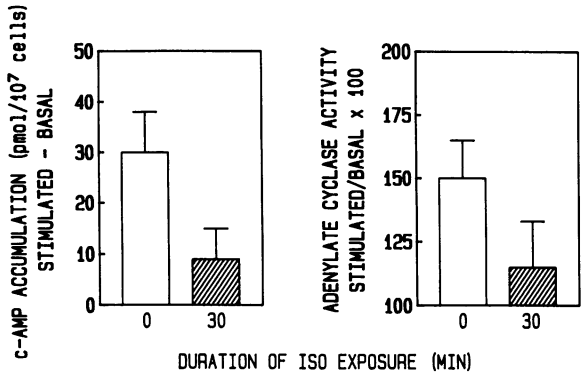
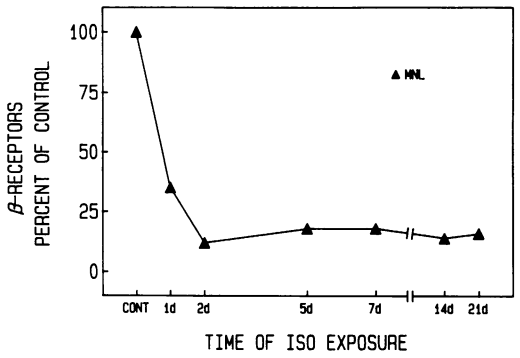


Figure 4. Desensitization of receptor function after isoproterenol. Animals were injected with 0.15 mg/kg isoproterenol and killed 30 min later. Receptor function was assessed as isoproterenol-stimulated cAMP accumulation in MNL and adenylate cyclase activity in heart. As basal levels of cAMP in MNL were low (typically < 20 pmols/10^7 cells), values were expressed as stimulated minus basal (mean±SEM, n = 3–5) ($P < 0.01$). Adenylate cyclase activity in sarcolemma was expressed as percentage of basal and is expressed as mean±SEM for three experiments ($P < 0.05$). Basal adenylate cyclase ranged from 110–160 pmol min^{-1} mg^{-1} protein. GTP (100 μM) was included in all incubations.



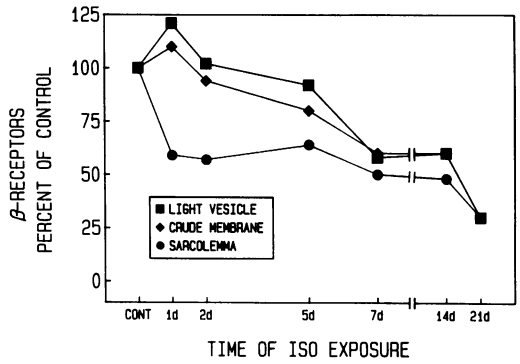


Figure 6. Time course of β-adrenergic receptor down-regulation in (*A*) MNL and (*B*) heart. Animals were infused with isoproterenol at 0.15 K_d mg/kg per h. At various times the animals were killed and the total number of receptors on MNL or heart was determined. All values are normalized to time zero. The K_d for MNL was 0.03±0.004 nM. For heart, we determined the number of receptors in crude membranes, light vesicles, and sarcolemmal. Each point represents the pooled blood or hearts from three animals and is representative of three to four experiments.

MNL during a 3-wk period that animals were infused with isoproterenol (0.15 mg/kg per h). Substantial down-regulation was observed within the first 24 h. This decrease in receptor number reached a plateau at 20% of control levels between 2 and 21 d. The affinity of the receptor for the ligand (K_d) did not significantly change after infusion of isoproterenol.

The sarcolemmal and light vesicle fractions that we isolate from guinea pig heart account for only about 30% of the total pool of cardiac β-adrenergic receptors (17). Thus, we also quantitated β-receptors in crude cardiac membranes, which would contain a more representative complement of cardiac β-receptors. Fig. 6 *B* demonstrates the kinetics of agonist-induced down-regulation of β-receptors on crude cardiac membranes, as well as sarcolemmal and light vesicle membranes, for a 3-wk period during which isoproterenol was infused via osmotic minipumps. Down-regulation of β-receptors in the crude cardiac membranes occurred after ≥ 2 days. The number of light vesicle β-adrenergic receptors decreased in parallel with the loss in β-adrenergic receptors in the crude cardiac membranes. The loss in surface sarcolemmal β-receptor occurred more rapidly but ultimately to a similar extent as the loss in light vesicle and crude cardiac membrane receptors. Thus, whereas isoproterenol administration produced a rapid sequestration of β-adrenergic receptors that occurred with similar rates in heart and MNL, isoproterenol-stimulated down-regulation of receptors occurred somewhat more slowly and to a lesser extent in the heart than in MNL.

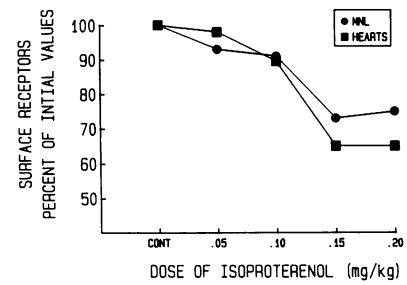


Figure 5. Changes in surface β-adrenergic receptor number in MNL and heart 1 h after an injection of four different doses of isoproterenol. Each point represents the pooled blood or hearts from three animals and is representative of two to three experiments.

Comparison of β-adrenergic receptor sequestration in heart and MNL after an acute pressure overload on the heart. The data presented so far indicate that redistribution of β-receptors on MNL and heart occurs similarly in response to high concentrations of circulating agonist. Concentrations of catecholamines are much higher at sympathetic nerve–effector cell synapses, and we wondered whether changes in synaptic levels of catecholamines would lead to receptor redistribution. To test this idea we created an acute increase in cardiac afterload by constricting the aorta. This increases the sympathetic drive and leads to the development of acute heart failure, with plasma norepinephrine levels nearly four times those of controls: 16,930±406 pg/ml (100 nM) vs. 4,772±457 (28 nM) ($P < 0.01$). The plasma norepinephrine level was high in control animals, presumably due to thoracotomy. The left ventricular end diastolic pressure was 18±6 mM Hg in pressure overloaded animals vs. 8±3 in sham-operated controls ($P < 0.05$). In animals with aortic constriction the number of surface β-receptors decreased an average of 9% on MNL and 61% in heart. In 7 of 10 animals, the loss of β-adrenergic receptors from the surface membrane was greater in heart than in MNL and overall the difference between heart and MNL was signifi-

cant with $P < 0.001$ by paired two-tailed t test. This substantial loss of sarcolemmal β-adrenergic receptors after aortic banding was accompanied by a 26% increase in the number of receptors present in the light vesicles. The K_d for [^{125}I]ICYP was similar in all groups of animals and in all membrane fractions. Corresponding to the decrease in β-adrenergic receptor with aortic construction, isoproterenol-stimulated adenylate cyclase activity was decreased by 17% in a crude membrane preparation ($n = 6$ for each group, $P < 0.05$). Pooled sarcolemma from three animals each ($n = 2$) revealed a decrease in isoproterenol-stimulated adenylate cyclase of 30%.

Discussion

Because MNL are easily accessible, assays of β-adrenergic receptors on human MNL have been used as a possible marker of receptors on less accessible tissues. Several studies demonstrate that changes in β-receptor number or response on MNL mirror those on other tissues (e.g., 10, 23–26). For example, recent data in 21 human subjects show a good correlation between total β-receptor number in membranes prepared from MNL and right atrial appendage (10). Comparisons between β-receptor expression on MNL and other tissues may be complicated because β-adrenergic receptors on MNL are exclusively of the β_2-subtype while receptors on other tissues such as heart, adipocytes, and kidney are predominantly of the β_1-subtype. The current study has used an animal model to examine possible differences in receptor compartmentation between MNL and the heart. Relatively little previous information has been available regarding β-adrenergic receptor compartmentation in tissues in vivo.

We had previously developed a method of studying redistribution of β-adrenergic receptors on intact human mononuclear leukocytes (MNL), and had found that although most of the receptors were redistributed after in vitro incubation of MNL with agonist, only minimal redistribution was observed in vivo after 30 min of exercise, 2 h of isoproterenol infusion, or 6 d of terbutaline (6, 12). Plasma catecholamine concentrations in these protocols did not reach the very high levels ($\geq$ 100 nM) seen in some clinical settings (i.e., pheochromocytoma, shock, or perhaps myocardial infarction), but these data suggested that redistribution of β-adrenergic receptors on MNL does not generally occur under physiological circumstances. These observations led us to question whether receptor redistribution on MNL accurately mirrors that of other cell types.

The method used in this study for assessing redistribution of β-adrenergic receptors on guinea pig MNL was similar to that used in previous studies with human MNL, and results after in vitro incubation with agonist were also similar. It is important to note that these methods employ intact MNL, while our methods for assessing redistribution of β-adrenergic receptors on guinea-pig heart involve subcellular fractionation of membrane preparations. With intact MNL, our methods measure two receptor compartments: receptors accessible to CGP-12177 (surface) and those that are not (sequestered). In contrast, we measure β-adrenergic receptors in three cardiac fractions: crude, sarcolemmal, and light vesicle. These methodologic differences do not permit an ideal comparison between MNL and heart. Another difference also hinders direct comparison between the two tissues. In studies with MNL, we are able to quantitate receptors as sites per cell. With receptor redistribution, therefore, the loss of surface receptors is reflected by an equal increase in the number of sequestered receptors. By contrast, in studies with the heart, we quantitate receptor number in each fraction as femtomoles per milligram of protein, even though the protein composition of the different fractions is likely to be quite different. Thus, when receptors are redistributed, the decrease in receptor density (femtomoles per milligram) in the sarcolemmal vesicles will not necessarily equal the increase in receptor density (femtomoles per milligram) in the light vesicles. To circumvent these two problems, we have focused our data analysis on the relative change in the number of receptors in the surface fractions in MNL and heart, as these are functionally active receptors.

In guinea pigs injected with isoproterenol, redistribution of β-adrenergic receptors occurred both in heart and MNL. Both the time course and the dose-response relationship were similar in the two tissues. The greater redistribution observed in guinea pig MNL relative to earlier in vivo studies with humans, probably reflects the greater dose of agonist administered. Likewise, agonist-induced receptor down-regulation was similar in MNL and heart, although it occurred faster and to a somewhat greater extent in MNL. In the heart this down-regulation was observed for light vesicles as well as sarcolemma β-receptors. The differences probably reflect more than just a difference between β_1- and β_2-receptors, inasmuch as other work from our laboratory has demonstrated that renal β_1- and β_2-adrenergic receptors down-regulate with similar kinetics in vivo (27, 28).

Except for the difference in the rate of down-regulation, the current data demonstrate that β-adrenergic receptors on MNL respond to circulating isoproterenol in a manner generally similar to that of receptors on the myocardium. Thus changes observed in the number, location, and function of β-adrenergic receptors produced by circulating nonselective agonists in human MNL are likely to reflect changes that also occur for β-adrenergic receptors in the heart (and perhaps other organs).

In contrast, we found aortic constriction selectively redistributed β-receptors in the heart but not MNL. Two factors are likely to account for this difference. First, increased sympathetic nervous system activity in the heart results in a concentration of norepinephrine at sympathetic nerve–cardiac cell synapses that can exceed plasma concentration by a factor of 1,000 (29). Receptors in MNL, in contrast, are exposed only to circulating catecholamines. Second, norepinephrine is selective for β_1- (heart) compared to β_2-receptors (MNL). The relative contribution of these two factors is not clear, although we suspect that the synaptic location of the norepinephrine rather than its β_1 selectivity is most important in this setting. The circulating norepinephrine concentration was in fact higher in the animals given an isoproterenol infusion (probably due to facilitory presynaptic β_2 receptors [30]) than in the animals with aortic constriction (Table I), yet far more sequestration of cardiac β-receptors occurred with the aortic constriction. The discrepancy between the 61% decrease in sarcolemmal β_1-receptors and the lesser decrease of isoproterenol-stimulated adenylate cyclase activity after aortic constriction might possibly be explained by enhanced coupling between a reduced number of receptors and the catalytic unit of adenylate cyclase. Since plasma norepinephrine levels were measured at only a single time point, we are unable to define possible differences in the kinetics of norepinephrine increase following aortic constriction and isoproterenol injection.

Table I. Pooled Results for Plasma Norepinephrine Levels, MNL, and Cardiac β-Adrenergic Receptors in Guinea Pigs Injected with Isoproterenol or in Acute Heart Failure

	Control	Aortic constriction for 1 h	Isoproterenol injection for 1 h
			(0.15 mg/kg)
Plasma norepinephrine level			
pg/ml	4,772±457 (n = 7)	16,930±406§ (n = 6)	23,211±798§ (n = 7)
MNL			
Surface sites			
Sites per cell	821±56 (n = 15)	748±112 (n = 10)	666±65* (n = 6)
% of control	100	92	81
Heart			
Sarcolemmal			
fmol/mg protein	108±10 (n = 12)	42±16§ (n = 10)	76±12‡ (n = 6)
% of control	100	39‖	71
Light vesicle			
fmol/mg	91±6 (n = 12)	115±14 (n = 8)	121±7 (n = 6)

The values represent mean±standard error. n ranges from 6 to 15 because measurements were not performed in all animals.
* $P < 0.05$ vs. control by paired t test.
‡ $P < 0.01$ vs. control by paired t test.
§ $P < 0.001$ vs. control by paired t test.
‖ $P < 0.001$ vs. MNL aortic constriction.

In conclusion, we have compared β-adrenergic receptor compartmentation in vivo in two tissues to determine whether measurements of MNL β-receptors are likely to be an accurate model for β-receptors in the heart. The data for animals subjected to aortic constriction demonstrate that MNL β-receptors do not reflect changes promoted by β_1-selective agonists and/or changes that occur at synapses where the catecholamine concentration greatly exceeds that to which MNL are exposed. However, data for animals administered isoproterenol clearly show that MNL and cardiac β-receptors respond very similarly to a circulating agonist that does not discriminate between β_1- and β_2-receptors. Under these circumstances MNL provide a useful model for β-adrenergic receptors on the heart and perhaps in other solid tissues as well.

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