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CHARACTERIZATION OF THE RAT MAST CELL BETA-ADRENERGIC RECEPTOR IN RESTING AND STIMULATED CELLS BY RADIOLIGAND BINDING

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Rat serosal mast cell beta-adrenergic receptors were characterized both functionally by assessing changes in histamine release and cyclic 3′,5′ adenosine monophosphate (cAMP) levels and directly by radioligand binding studies using [^{3}H]dihydroalprenolol ([^{3}H]DHA), a beta-adrenergic antagonist. Mast cells were obtained by lavage of the pleural and peritoneal cavities of Sprague-Dawley rats and were purified on metrizamide gradients to greater than 95% purity. Resting mast cells stimulated with beta-adrenergic agonists demonstrate a marked rise in cAMP levels after a 15-sec incubation. However, the same concentrations of these agonists have no effect on IgE-mediated mast cell histamine release.

[^{3}H]DHA binding to intact mast cells is rapid, reversible, saturable, and stereoselective. The cells possess 40,000 ± 14,000 beta-adrenergic receptors/cell and demonstrate a binding affinity of 1.58 ± 0.56 nM for [^{3}H]DHA. Competition studies reveal that 83.5% of the receptors are of the beta$_2$ subtype and 16.5% are beta$_1$. Neither sensitization with anti-DNP-BSA IgE nor subsequent challenge with specific antigen alters mast cell beta-adrenergic receptor characteristics. Rat mast cells possess large numbers of high affinity beta-adrenergic receptors, primarily of the beta$_2$ subtype, coupled to adenylate cyclase, but the role of these receptors in mast cell secretory events is not yet established.

The presence of beta-adrenergic receptors on mast cells has been suggested by the inhibition of immunologic histamine release from human lung fragments incubated with isoproterenol (1) and from purified human lung mast cells incubated with fenoterol, a beta$_2$-specific agonist (2). The coupling of this receptor to adenylate cyclase has been postulated in many cell types (3), and the changes in rat peritoneal mast cell cyclic 3′,5′ adenosine monophosphate (cAMP)[3] levels induced by beta-adrenergic agents support this premise (4, 5). With the advent of specific radiolabeled beta-adrenergic antagonists and the methods of radioligand binding, it is now possible to directly assess beta-adrenergic receptor numbers and binding affinities in cells and tissues and to observe the functional changes that stimulation or blockade of these receptors elicit. Therefore, we chose to characterize the rat serosal mast cell beta-adrenergic receptor both functionally, by investigating changes in mediator release and cAMP metabolism, and also by direct radioligand binding, utilizing [^{3}H]DHA, a potent beta-adrenergic antagonist. Furthermore, we used these methods to study the effects of specific IgE antibody sensitization and subsequent antigen challenge on the rat mast cell beta-adrenergic receptor.

MATERIALS AND METHODS

Chemicals. The following materials were purchased from the manufacturer: heparin, histamine, propanolol, catechol, aminophylline, epinephrine bitartrate, norepinephrine, isoproterenol (Sigma, St. Louis, MO); DNAse, ascorbic acid (Calbiochem, La Jolla, CA); cAMP radioimmunoassay kit (Collaborative Research, Waltham, MA); 1(propyl-2,3-^{3}H) dihydroalprenolol 42.5 Ci/mmol (Amersham, Arlington Heights, IL); Whatman GF/C 2.4-cm glass fiber filters (Fisher Scientific, Pittsburgh, PA); BetaPhase liquid scintillation fluid (Westchem, San Diego, CA); gelatin (Difco Labs, Detroit, MI); metrizamide, analytical grade (Accurate Chemical and Scientific Corp., Hicksville, NY).

The following materials were generously donated: phentolamine, terbutaline (Ciba-Geigy, Summit, NJ); fenoterol (Boehringer Ingelheim, Ridgefield, CT); atenolol (Stuart Pharmaceuticals, Wilmington, DE); butoxamine (Burroughs Wellcome, Research Triangle Park, NC); L- and D-propranolol (Ayerst, New York, NY); mouse hybridoma anti-DNP-BSA IgE antibody and DNP-BSA antigen (Dr. Futong Lu and Dr. David Katz, La Jolla, CA).

Mast cell purification. Sprague-Dawley rats (200 to 250 gm) were sacrificed by decapitation; the pleural and peritoneal cavities were lavaged with Tyrode's buffer lacking divalent cations and containing 0.1% gelatin and 8500 U/liter heparin. The resulting cell suspension was centrifuged at 200 × G for 20 min to pellet the mast cells, erythrocytes, and mononuclear cells. The mast cell population was purified on metrizamide gradients by a method described previously (6) and was found to be greater than 95% pure and viable by trypan blue exclusion.

Histamine release experiments. Mast cells (10^6/ml) were sensitized with 1 μg anti-DNP-BSA antibody for 30 min at 37°C in Tyrode's buffer without Ca^{++} and Mg^{++} and with 0.1% gelatin and 20 mg/liter DNAse. The sensitized cells were washed twice and resuspended in Tyrode's buffer (containing 1.8 mM Ca^{++} and 1 mM Mg^{++}) and then challenged with DNP-BSA antigen (100 ng/3 × 10^5 cells) for 10 min at 37°C. The cell suspension was centrifuged at 200 × G for 10 min to pellet the cells, and both the supernatant and pellet were saved to determine the percentage of histamine release. Net histamine release was determined by bioassay on the isolated, atropinized guinea pig ileum (7) and was verified by a radioenzyme assay (8). Background histamine release was generally less than 5% of total.

cAMP experiments. To assess resting cell cAMP levels, 10^6 mast cells/tube in a vol of 180 μl were incubated with beta-adrenergic agonists for 0.25, 1, or 5 min at 37°C. The reactions were quickly stopped by the addition of 20 μl cold 100% TCA, followed by immediate freezing in dry ice and acetone. The reaction mixtures were then thawed on ice, sonicated with a Branson Sonifier, and centrifuged at 250 × G for 20 min at 4°C, and the supernatant was decanted, extracted three times with water-saturated ether, lyophilized, and resuspended in 100 μl of cAMP buffer. The level of cAMP was determined employing a ^{125}I radioimmunoassay.

Radioligand binding experiments. Mast cells (1.0 to 1.5 × 10^5/tube) were incubated in polypropylene tubes with [^{3}H]DHA and either buffer alone or 1 μM propranolol at 37°C usually for 20 min. The incubation buffer consisted of calcium-free Tyrode's buffer with 0.1% gelatin, DNAse 20 mg/liter, 10^{-4} M phentolamine (to decrease nonspecific binding); in competition experiments, 10^{-3} M ascorbic acid and 3 × 10^{-4} M catechol were added to stabilize the sympathomimetic agents. Final pH was adjusted to 7.8. At the end of the incubation period, 5 ml of buffer at room temperature were added to each tube, and the binding was terminated by rapid vacuum

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[3] Abbreviations used in this paper: cAMP, cyclic 3′,5′ adenosine monophosphate; [^{3}H]DHA, 1(propyl-2,3-^{3}H) dihydroalprenolol; K_1, rate of association; K_2, rate of dissociation; K_D, equilibrium dissociation constant; B_{max}, receptor number; GTP, guanosine triphosphate.

filtration through GF/C glass fiber filters. The tubes were washed with another 5 ml of incubation buffer, which was similarly filtered. The filters were briefly air dried and placed in scintillation vials; 4 ml of scintillation fluid was added; and after at least 1 hr of equilibration, the radioactivity was counted in a Beckman LS 230 liquid scintillation counter with 40% efficiency. Specific binding is defined as the difference in counts per minute in binding in the absence and presence of 1 μM propranolol and was generally 40 to 60% of total binding.

Analysis of data. The results described represent the mean ± standard deviation unless otherwise indicated. Statistical significance was determined using the paired two-tailed Student's *t*-test.

RESULTS

Effect of beta-adrenergic agents on cAMP levels. Rat serosal mast cells (10^6/tube) were incubated for 15 sec, 1 min, and 5 min with 10^{-6} M, 10^{-5} M, and 10^{-4} M isoproterenol to assess the effects of beta-adrenergic stimulation on resting cell cAMP levels. By 15 sec of incubation, there was a dose-dependent, significant increase in mast cell cAMP levels ($p < 0.025$) induced by all concentrations of isproterenol up to 250% of baseline (Fig. 1). This elevation in cAMP nearly disappeared by 1 min, although the levels remained slightly above control values for up to 5 min after the addition of the agonist. Similar early augmentation of resting cell cAMP levels was observed utilizing fenoterol or terbutaline as the beta-adrenergic agonist, with maximum increases to 158 and 188% of control values with 10^{-4} M fenoterol and 10^{-3} M terbutaline, respectively.

Effect of beta-adrenergic agents on histamine release. After a 15-sec or 5-min preincubation with buffer or isoproterenol (10^{-6} to 10^{-4} M) or fenoterol (10^{-8} to 10^{-4} M), 3×10^5 mast cells sensitized with anti-DNP-BSA IgE antibody were challenged with DNP-BSA antigen for 10 min to determine the effect of beta-adrenergic agonists on mast cell mediator release. At the concentrations studied, neither fenoterol nor isoproterenol significantly affected antigen-induced histamine release ($p > 0.20$) (Fig. 2), although these concentrations were sufficient to markedly change mast cell cAMP levels.

[^{3}H]DHA binding to the beta-adrenergic receptor. To assure that radioligand binding is to a cell surface receptor, binding must be linear with increasing cell number, reversible, saturable, and stereoselective. Specific [^{3}H]DHA binding to intact mast cells after a 20-min incubation at 37°C was linear, with cell numbers in a range from 0.5 to 8.0 × 10^5 cells/tube (r =

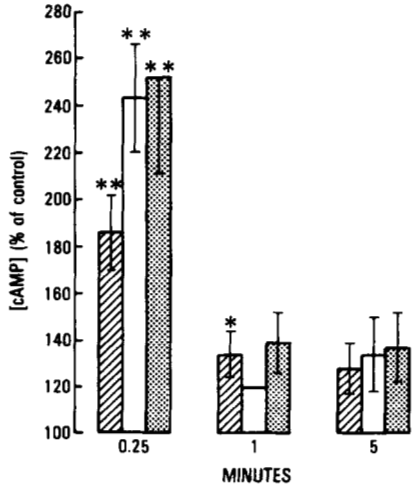

Figure 1. Effect of isoproterenol on resting mast cell cAMP levels. Isoproterenol 10^{-6} M ▨, 10^{-5} M □, and 10^{-4} M ▩ were incubated with 10^6 mast cells/tube for the times shown, and cAMP levels were measured. Values shown are the mean ± SE of duplicate determinations from 2 or 3 experiments. Results significantly different statistically from controls are indicated by * ($p < 0.05$) and ** ($p < 0.025$).

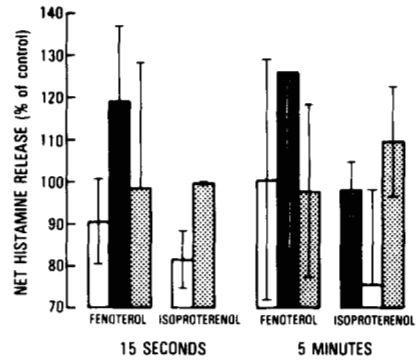

Figure 2. Effect of beta-adrenergic agonists on antigen-induced mast cell histamine release. Net histamine release (expressed as percent of control values) from cells incubated with 10^{-8} M □, 10^{-6} M ■, or 10^{-4} M ▩ fenoterol or 10^{-6} M ■, 10^{-5} M □, or 10^{-4} M ▩ isoproterenol for 15 sec or 5 min is depicted as the mean ± SE of duplicate determinations from 2 or 3 experiments. Spontaneous histamine release was 4% and antigen-stimulated histamine release was 25% of total cell histamine.

0.996). The rate of association (on rate, K_1) of [^{3}H]DHA binding to rat mast cells was determined by incubating 1×10^5 cells/tube with [^{3}H]DHA and either buffer or 1 μM propranolol for various time periods and then stopping the reactions by rapid vacuum filtration. Binding at 37°C was rapid and complete by 15 to 20 min for all concentrations of [^{3}H]DHA used in these experiments (Fig. 3). Therefore, 20 min was used as the equilibrium time point in subsequent experiments. The reversibility of the system, expressed as the rate of dissociation (off rate, K_2), was demonstrated by bringing the cells to equilibrium with [^{3}H]DHA for 20 min at 37°C, followed by the addition of 1 μM propranolol. Specific radioligand binding was displaced in a time-dependent manner, with total displacement by 15 to 20 min (Fig. 4). The K_2, determined by calculating the slope of the percentage of specific [^{3}H]DHA bound plotted semilogarithmically *vs* time, is 0.106 ± 0.023 min^{-1}, while the K_1, calculated from the method described by Williams and Lefkowitz (9), is 0.075 nM^{-1} min^{-1}. The equilibrium dissociation constant (K_D), equal to K_2/K_1, is 1.4 nM, as determined by the on and off rates.

Scatchard analysis of equilibrium binding data. Several concentrations of [^{3}H]DHA (0.21 to 9.00 nM) were incubated with 1.0×10^5 mast cells and either buffer or 1 μM propranolol for 20 min to prove that beta-adrenergic binding to rat mast cells is saturable. At [^{3}H]DHA concentrations greater than approximately 4 nM, specific [^{3}H]DHA binding levels off or saturates (Fig. 5). To linearize this equilibrium binding data and determine the number and binding affinity of the beta-adrenergic receptors, Scatchard analysis was performed (10) (Fig. 6), revealing one population of high affinity beta-adrenergic receptors with a K_D of 1.58 ± 0.56 nM and a receptor density, or B_{max}, of 6.64 ± 2.32 fmol/10^5 cells, indicative of 40,000 ± 14,000 beta-adrenergic receptors/mast cell. Nearly identical Scatchard plots were obtained using sonicated rat mast cell membrane preparations instead of intact cells.

Stereoselectivity of the beta-adrenergic receptor. The D and L enantiomers of propranolol were utilized as competitors to demonstrate the stereoselectivity of mast cell beta-adrenergic receptor binding. Mast cells were incubated with [^{3}H]DHA and several concentrations of each of the propranolol stereoisomers to determine the concentrations of the competitor needed to inhibit specific [^{3}H]DHA binding. As shown in Figure 7, L-propranolol was 52 times more potent in competing for beta-adrenergic binding sites than D-propranolol, with inhibitory constants (K_i) of 0.44 nM and 23.1 nM, respectively.

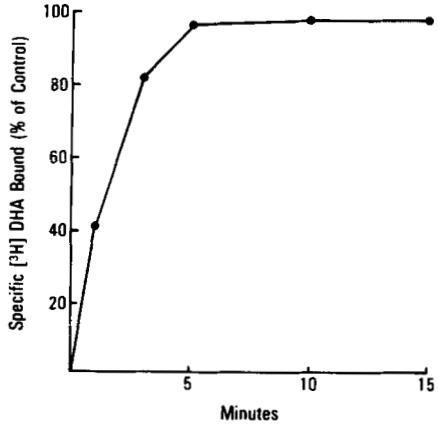

Figure 3. Rate of association of [^{3}H]DHA binding to rat mast cells. Incubations of 10^5 mast cells/tube and 0.7 to 2.80 nM [^{3}H]DHA were performed at 37°C for the times shown. In this representative experiment, one of seven similar experiments, each done in duplicate, 2.58 nM [^{3}H]DHA was used, and specific binding was determined in the absence and presence of 1 μM propranolol. Nonspecific binding did not change appreciably during the 20-min period.

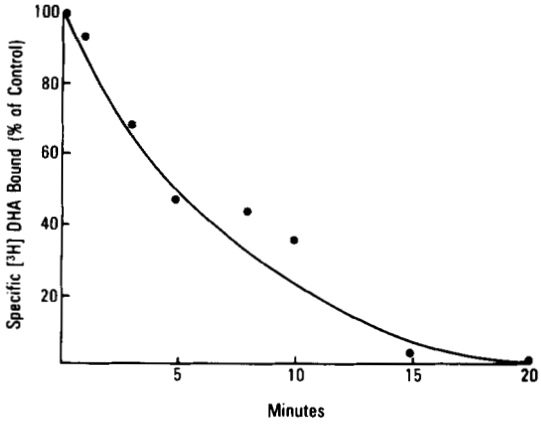

Figure 4. Rate of dissociation of [^{3}H]DHA binding to rat mast cells. Mast cells (10^5/tube) were equilibrated with 2.68 nM [^{3}H]DHA for 20 min, shown here as 100% specific [^{3}H]DHA bound at time 0. Times shown are after the addition of 1 μM propranolol. This representative experiment (one of four similar experiments) was performed in triplicate.

Competition experiments. Several agents have been shown to interact at beta-adrenergic receptors to various degrees. To assess this interaction, competition experiments were performed utilizing adrenergic agonists and antagonists (Table I). As expected for a beta-adrenergic receptor, isoproterenol (K_i = 5.96 μM) was much more potent than epinephrine (K_i = 24.0 μM) and norepinephrine (K_i = 546 μM) in competing for beta binding sites. Fenoterol and terbutaline demonstrated K_i at the beta-adrenergic receptor of 21.0 and 16.1 μM, respectively, a result consistent with those reported in other cells (11). Intact cells tend to demonstrate higher K_i values for agonists than cell membranes or tissues, however, presumably because of the presence of GTP in intact cells, which may convert high affinity binding sites to lower affinity. Aminophylline, with a K_i greater than 2 mM, has very little effect on [^{3}H]DHA binding. The nonspecific beta-adrenergic antagonist, propranolol, displayed a K_i of 1.6 nM, while butoxamine, a $beta_2$-selective agent *in vivo* but nonselective for beta-adrenergic subtypes in *in vitro* binding studies, demonstrated a K_i of 1.90 μM, again consistent with reports in other tissues (11).

The fact that epinephrine is more potent than norepinephrine in inhibiting [^{3}H]DHA binding to rat mast cells suggests that the cells possess primarily receptors of the $beta_2$ subtype. To precisely quantitate the percentage of $beta_1$- and $beta_2$-adrenergic receptors on these cells, the specific $beta_1$-adrenergic receptor antagonist, atenolol, was used in competition studies. Atenolol binds to $beta_1$ receptors with 50 times greater affinity than $beta_2$ receptors, and the differences in these binding affinities can be used to directly quantitate receptor subtypes. The resulting competition curves demonstrated two slopes with K_D of 0.428 μM and 68.1 μM, indicating the presence of both $beta_1$ and $beta_2$ subtypes (Fig. 8). Computer-aided iterative graphic analysis of these curves (12) reveals that rat serosal mast cells possess 16.5% $beta_1$- and 83.5% $beta_2$-receptor subtypes, with the curve fitted to two binding sites significantly better than one ($p = 0.005$).

Effect of sensitization and challenge on beta receptor binding. Purified rat mast cells: 1) were incubated with complete Tyrode's buffer alone; 2) were sensitized for 30 min at 25°C with anti-DNP-BSA IgE; or 3) were sensitized and then challenged with DNP-BSA antigen at 37°C, followed by standard

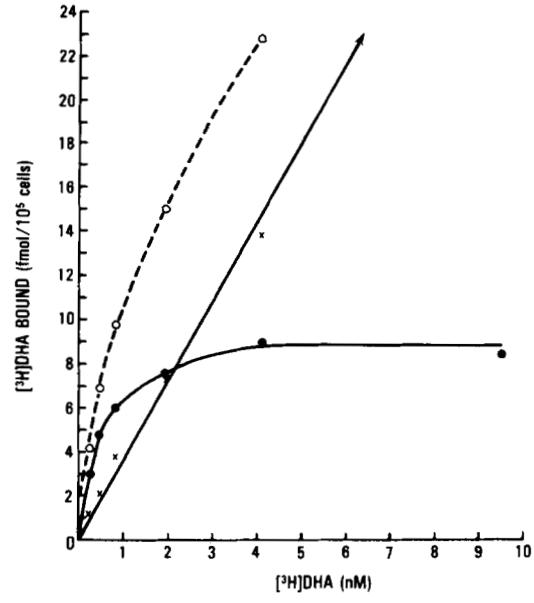

Figure 5. Saturation of [^{3}H]DHA binding to rat mast cells. Intact rat mast cells were incubated with increasing concentrations of [^{3}H]DHA for 20 min at 37°C, and total (○), specific (●), and nonspecific (×) binding were determined as described in *Materials and Methods*. Data shown are the mean of triplicate determinations and are representative of four similar experiments.

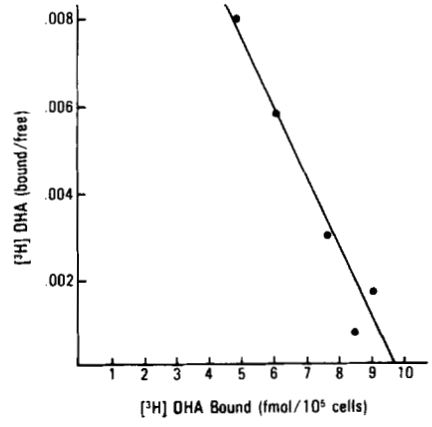

Figure 6. Scatchard analysis of [^{3}H]DHA binding to rat mast cells. The equilibrium binding data from five experiments performed in triplicate were analyzed according to the method of Scatchard, revealing a K_D (mean ± SD) for [^{3}H]DHA binding of 1.58 ± 0.56 nM and a B_{max} (mean ± SD) of 6.64 ± 2.32 fmol/10^5 cells. This representative experiment demonstrates the linearity of the relationships.

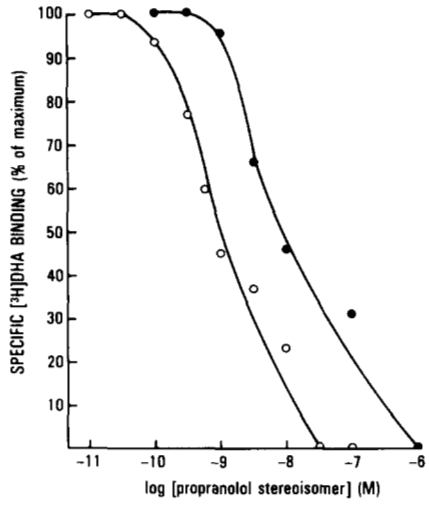

Figure 7. Stereoselectivity of [^{3}H]DHA binding to rat mast cells. D (●) and L (○) propranolol were used in 20-min incubations to compete for [^{3}H]DHA binding sites on rat mast cells. Values shown are the mean of results from four experiments, each performed in triplicate.

TABLE I

K_i values for beta-adrenergic agents[a]

Agonist	K_i[b]	Antagonist	K_i
Isoproterenol	5.96 ± 2.8 μM	Propranolol	1.6 ± 0.7 nM
Terbutaline	16.1 ± 0.6 μM	Butoxamine	1.90 ± 1.2 μM
Fenoterol	21.0 ± 15.4 μM		
Epinephrine	24.0 ± 8.3 μM		
Norepinephrine	546 ± 145 μM		
Aminophylline	>2 mM		

[a] Several concentrations of each agent were incubated with 10^5 rat mast cells and 1.45 to 2.55 nM [^{3}H]DHA in competition experiments, and percent inhibition of specific binding was determined.

[b] $K_i = (K_D)(I_{50})/K_D + (\text{ligand})$, where K_D is the equilibrium dissociation constant of [^{3}H]DHA binding to rat mast cells, I_{50} is the concentration of competitor required to inhibit 50% of specific [^{3}H]DHA binding, and (ligand) is the concentration of [^{3}H]DHA used in a particular experiment. Values shown are means ± SD of at least three experiments performed in duplicate.

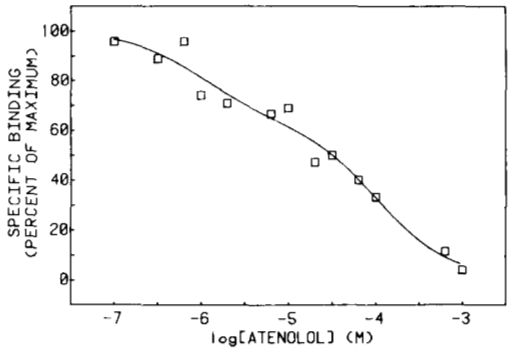

Figure 8. Quantitation of beta-adrenergic subtypes on rat mast cells. Several concentrations of atenolol were used to compete for specific [^{3}H]DHA binding sites. Points represent the means of duplicate determinations in this representative experiment. Four similar experiments were performed, and receptor subtype calculations are based on computer analysis of the combined data (see text).

[^{3}H]DHA binding for 20 min with and without 1 μM propranolol. Scatchard analysis of equilibrium binding data was performed to identify any changes in receptor number or binding affinity produced by sensitization or challenge. Both cells sensitized with IgE and those subsequently challenged with antigen showed minor changes in receptor numbers that were not statistically significant ($p > 0.20$ in each case), compared to control values, and showed an insignificant change in binding affinity ($p > 0.20$ in each case) as assessed by K_D (Fig. 9). Thus, no effect of IgE/antigen-stimulated mediator release on mast cell beta-adrenergic receptor characteristics was demonstrated.

DISCUSSION

These experiments were undertaken in an attempt to clarify the relationship between adrenergic stimuli and mast cell function. The importance of adrenergic phenomena in allergic diseases has been investigated extensively. Some studies have shown a hyporesponsiveness to beta-adrenergic stimuli in atopic subjects compared to normals (13); also, an imbalance in the ratios of alpha- and beta-adrenergic receptors has been postulated in the pathophysiology of asthma (14). On the other hand, the final contribution of the mast cell and its mediators to producing allergic reactions is still not fully defined, although the *in vivo* effects of histamine, prostaglandins, and leukotrienes suggest a prominent role for this cell (15).

Studies of the complex biochemistry of mast cell mediator release have highlighted the importance of calcium fluxes (16), arachidonic acid metabolism (17), phospholipid methylation (18) and turnover (19), and cAMP metabolism (20) in the release process. cAMP levels are regulated both by adenylate cyclase and phosphodiesterase activity, and for beta-adrenergic stimulation to affect mast cell secretory events, there must be occupancy of a regulatory unit by GTP (21) and subsequent coupling to a cAMP-dependent protein kinase (22), which in turn facilitates mediator release. Beta-adrenergic receptors in many cells and tissues have been shown to be coupled to adenylate cyclase (3), and this is underlined in the present studies, where stimulation of mast cell beta-adrenergic receptors with specific agonists results in rapid and pronounced changes in resting cell cAMP levels (Fig. 1). Since the forward rate of receptor occupancy is dependent upon the concentration of agonist or antagonist added, it is not unreasonable for 10^{-6} to 10^{-4} M isoproterenol to elicit functional changes in mast cell biochemistry in a matter of seconds.

Previous investigators have been unable to demonstrate a reproducible inhibition of histamine release from rat mast cells preincubated with beta-adrenergic agonists (23, 24), in spite of the observed cAMP changes. With both the nonselective agent, isoproterenol, and the beta$_2$-specific agonist, fenoterol, no significant changes in antigen-induced mast cell mediator release were observed after 15-sec and 5-min preincubations (Fig. 2). Preliminary studies of purified human lung mast cells reveal a moderate inhibition of mediator release by fenoterol (2), which suggests some important biochemical differences in mast cells from different species and different sites.

Direct radioligand binding studies were performed to correlate the functional changes in mast cells exposed to adrenergic

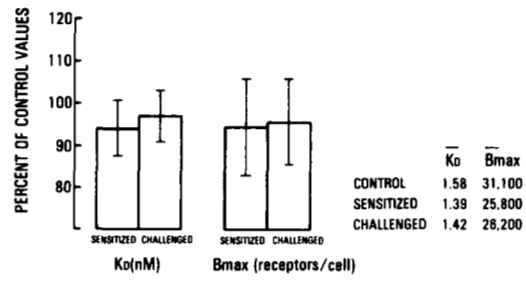

Figure 9. Changes in mast cell beta-adrenergic receptor density (B_{max}) and binding affinity (K_D) with IgE sensitization and subsequent antigen challenge. Results are means ± SD of values from four experiments done in duplicate and are depicted as percent of control (nonsensitized cells) values. Also shown are means of the K_D and B_{max} for each group.

agents with a quantitative assessment of beta-adrenergic receptors on the mast cell. That direct radioligand binding of [^{3}H]DHA to rat mast cells is indeed to beta-adrenergic receptors was confirmed by several experiments. Specific binding was linear with mast cell number in a range where less than 10% of the radioactivity added was bound. [^{3}H]DHA binding was rapid at 37°C (Fig. 3) and completely reversible upon addition of 1 μM propranolol (Fig. 4), displaying an equilibrium dissociation constant of 1.4 nM calculated from the on and off rates. Beta-adrenergic antagonist binding to the cells was saturable (Fig. 5) with a K_D of 1.58 ± 0.56 nM derived from Scatchard analysis and a B_{max}, or receptor density, of 40,000 ± 14,000 receptors/cell (Fig. 6). The nearly identical numbers for the equilibrium dissociation constant determined in two different ways confirms the consistency of the experimental methods. The linearity of the Scatchard plot indicates the presence of a single population of high-affinity beta-adrenergic binding sites on rat mast cells. The clear stereoselectivity of the system (Fig. 7) argues against the possibility that simple uptake of the radioligand by the cells could be confused for receptor binding. The 50-fold increased potency of the L stereoisomer of propranolol has been seen in several other binding studies and in other rat tissues (25–27).

Specific adrenergic agonists and antagonists have been used in competition experiments to classify beta-adrenergic receptor subtypes. The observed order of potency of isoproterenol>terbutaline>epinephrine>>norepinephrine (Table I) is most consistent with binding primarily to $beta_2$ receptor subtypes. To further substantiate this assertion, competition curves utilizing specific $beta_1$- or $beta_2$-adrenergic ligands are required. Although fenoterol and terbutaline are relatively $beta_2$-specific *in vivo*, this specificity has been absent in radioligand binding studies (11). However, atenolol, a $beta_1$-adrenergic antagonist, has been shown to bind to $beta_1$-adrenergic receptors in various tissues, with K_D ranging from 0.53 to 1.70 μM, and to bind less avidly to $beta_2$-adrenergic receptors, with K_D in a range from 14 to 30 μM, thus allowing differentiation of the $beta_1$ and $beta_2$ receptor subtypes (11). Experiments utilizing atenolol to compete for [^{3}H]DHA binding sites revealed competition curves with two distinct slopes, with K_D of 0.428 and 68.1 μM (Fig. 8). Computer analysis of the curves demonstrated that 83.5% of the rat mast cell binding sites were $beta_2$ and 16.5% $beta_1$. This is not an entirely novel finding, as other cells have been shown to possess both subtypes of beta-adrenergic receptors coupled to adenylate cyclase (28). However, it raises many questions as to the functional significance of the two receptor subtypes, which we are currently attempting to address in ongoing studies.

The effects of mast cell activation and secretion on beta-adrenergic receptor numbers has been of particular interest for two reasons. First, beta-adrenergic receptor binding sites have been identified on the secretory granules of rat peritoneal mast cells (29), which suggests possible changes in receptor characteristics when these granules are exposed. Second, phospholipid methylation takes place quickly after mast cell stimulation (18), and in the rat reticulocyte, phospholipid methylation (which in some cells is stimulated by beta agonists) (30) unmasks cryptic beta-adrenergic receptors, possibly by changing membrane fluidity and surface characteristics (31). Therefore, antigen stimulation of the sensitized mast cell could produce alterations in the surface beta-adrenergic properties. However, our data demonstrate that neither the number of beta-adrenergic receptors nor their binding affinities change during sensitization or 20 min after challenge with specific antigen (Fig. 9). This does not preclude the possibility of a transient change in receptor numbers that cannot be identified by binding experiments, late changes in receptor characteristics, or even destruction of some surface receptors by mast cell proteases released during the secretory process.

Thus, rat mast cells possess large numbers of high affinity beta-adrenergic receptors predominantly of the $beta_2$ subtype, which, through their modulation of adenylate cyclase, may play an intricate part in the biochemistry of this cell but which do not appear to directly regulate IgE-mediated histamine release.

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