Selectivity of Dobutamine for Adrenergic Receptor Subtypes

IN VITRO ANALYSIS BY RADIOLIGAND BINDING

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ABSTRACT The cardiovascular responses elicited by dobutamine are distinctly different from those produced by other adrenergic or dopaminergic agonists. To test the hypothesis that dobutamine could have differential affinities for adrenergic receptor subtypes, and that such subtype selectivity could be related to its relatively unique pharmacologic properties, we assessed the ability of dobutamine to displace adrenergic radioligands from membrane receptors in a number of tissues of previously characterized adrenergic receptor subtype. For beta adrenergic receptors identified by (−) [^{3}H]dihydroalprenolol (DHA), dobutamine had significantly greater affinity for the β_1 subtype (K_D = 2.5 μM in rat heart and 2.6 μM in turkey erythrocyte) than for the β_2 subtype (K_D = 14.8 μM in frog heart and 25.4 μM in rat lung) ($P < 0.001$). For alpha adrenergic receptors, dobutamine had markedly greater affinity for the α_1-subtype identified by [^{3}H]prazosin (K_D = 0.09 μM in rat heart and 0.14 μM in rabbit uterus) than for the α_2-subtype identified by [^{3}H]dihydroergocryptine (DHE) (K_D = 9.3 μM in human platelet) or by [^{3}H]-yohimbine (K_D = 5.7 μM in rabbit uterus) ($P < 0.001$).

Like other β_1-agonists, in the absence of guanine nucleotide, dobutamine competition curves for DHA binding in rat heart demonstrated two classes of binding sites, with one site of significantly higher affinity (K_D = 0.5 μM, P = 0.008) than the single class of binding sites (K_D = 5.2 μM) identified in the presence of guanine nucleotide. However, unlike β_2- or α_2-agonists, dobutamine displacement of DHA binding in rat lung or of DHE binding in human platelets demonstrated only a single class of binding sites, and guanine nucleotide had only minimal effects.

We conclude that dobutamine is selective for β_1 as opposed to β_2, and for α_1 as opposed to α_2 adrenergic receptors. Furthermore, guanine nucleotide effects on dobutamine binding, and biochemical response data in vitro suggest that dobutamine is a β_1-agonist, but has little intrinsic activity at β_2 and α_2-receptors. This selectivity for adrenergic receptor subtypes may be part of the basis for dobutamine's distinctive pharmacologic properties in vivo.

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INTRODUCTION

The pharmacologic effects produced by infusions of the synthetic catecholamine dobutamine differ from those elicited by other adrenergic agonists. At doses producing equivalent enhancement of cardiac inotropy, dobutamine induces a smaller positive chronotropic effect than isoproterenol. In addition, dobutamine induces neither the vasodilatory effects of isoproterenol or epinephrine, which are mediated through vascular beta adrenergic receptors, nor the vasoconstrictive effects of norepinephrine on arteriolar resistance vessels, mediated through vascular alpha adrenergic receptors. Furthermore, dobutamine is reportedly devoid of the presynaptic effects on endogenous norepinephrine release exhibited by its parent compound dopamine and by other adrenergic agonists (1–4).

The pharmacologic properties of dobutamine that account for its unique spectrum of hemodynamic effects have not been previously elucidated. One tenable hypothesis is that dobutamine may bind selectively to specific subtypes of alpha and beta adrenergic receptors, and that such selective binding could be causally related to its distinctive pharmacologic effects. The original division of biologic responses to catecholamines into two categories, alpha and beta, based on the relative potencies of adrenergic compounds in eliciting (or blocking) specific physiologic effects (5) has been

extended in recent years by further subclassification of both beta adrenergic (6) and alpha adrenergic (7) responses. Since current concepts of adrenergic agonist and antagonist action suggest that the specificity of biologic responses to adrenergic agents resides in the nature of the sarcolemmal adrenergic receptors, radioligand binding studies have proven to be a useful complement to physiologic measurements in determining the subclassification of adrenergic receptors (8–11). Furthermore, recent insights into the important regulatory function of guanine nucleotides in the coupling of adrenergic agonist binding to the subsequent biochemical and physiologic sequelae of receptor activation have expanded the role of radioligand binding methods in assessing the molecular pharmacology of adrenergic compounds. At least for β_1-, β_2-, and α_2-receptors, the ability to form a high affinity ligand-receptor complex which is shifted to a lower affinity state by guanine nucleotides is directly correlated with the intrinsic activity (i.e., the maximum response produced by saturating concentrations of agonist) of adrenergic agonists in eliciting biochemical or physiologic responses. Guanine nucleotides produce a large shift in apparent affinity for full agonists and a smaller shift for partial agonists. Competitive binding curves for antagonist compounds are unaffected by guanine nucleotides (12–14).

To test the hypothesis that dobutamine may bind selectively to specific subtypes of alpha and beta adrenergic receptors, we have assessed the ability of dobutamine to displace tritiated adrenergic ligands from both alpha and beta adrenergic receptors in a number of tissues in which the adrenergic receptor subtype specificity has been previously defined. In addition, as an indirect assessment of the specific activity of dobutamine for activation of responses mediated through specific adrenergic receptor subtypes, we have assessed the effect of guanine nucleotides upon dobutamine binding in these selected tissues.

METHODS

Preparation of membrane homogenates. Rat cardiac membranes were prepared as described in previous publications from our laboratory (15), and frog heart membranes were prepared in an identical fashion. Membrane fractions from human platelet, rabbit uterus, rat lung, and turkey erythrocyte were prepared with minor modifications of previously published techniques (10, 16–18).

Radioligand binding experiments. In each radioligand binding experiment, aliquots of a given membrane preparation were incubated with a fixed concentration of radioligand in the presence of varying concentrations of dobutamine ranging from 1 nM to 1 mM. In addition, in each experiment, radioligand binding in the absence of added dobutamine, and in the presence of either 10 μM (±) propranolol (β-receptors except lung), 10 μM (±) propranolol (lung β-receptors), or 10 μM phentolamine (α-receptors) was used to define maximal binding and nonspecific binding, respectively.

The non-subtype selective antagonist (−) [^{3}H]dihydroalprenolol (DHA)[1] (New England Nuclear, Boston, Mass.; specific activity 48 Ci/mmol) was utilized to identify β-adrenergic receptors in membranes derived from rat heart, frog heart, turkey erythrocyte, and rat lung. The non-subtype selective antagonist [^{3}H]dihydroergocryptine (DHE) (New England Nuclear; 33 Ci/mmol sp act) was used to identify α-adrenergic receptors in human platelets and rat heart. We also used the α_1-specific antagonist [^{3}H]prazosin (Pfizer, Sandwich, Kent, England; 33 Ci/mmol sp act) to label α_1-receptors in rat heart and rabbit uterus, and the α_2-specific antagonist [^{3}H]yohimbine (New England Nuclear; 81.4 Ci/mmol sp act) to label α_2-receptors in rabbit uterus.

Incubations were performed in the following respective concentrations of Tris HCl and $MgCl_2$: 50 mM, 16.7 mM, pH 7.4 (rat heart, frog heart); 60 mM, 20 mM, pH 7.5 (turkey erythrocyte); 40 mM, 5 mM, pH 8.0 (rat lung); 33.3 mM, 6.7 mM, pH 7.5 (rabbit uterus); 40 mM, 11 mM, pH 7.4 plus EDTA 2.5 mM (human platelet). Incubations were conducted for 20 min at 25°C, with two exceptions: turkey erythrocyte membranes (20 min at 30°C) and human platelet membranes (30 min at 25°C). The effects of guanine nucleotides were assessed by adding 0.1 mM (final concentration) guanyl-5′-yl imidodiphosphate (GPP). We concluded the incubations by rapid vacuum filtration of the entire assay mixture over Whatman GFC glass fiber filters (Whatman, Inc., Clifton, N. J.) followed by rapid washing of the filters with 15–20 ml incubation buffer at 0°C. Filters were placed in a Triton X-100-toluene based fluor, and radioactivity was determined in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Other incubation conditions and binding characteristics of each membrane-radioligand system we employed are summarized in Table I.

The saturability, reversibility, stereospecificity, and adrenergic receptor subtype specificity of radioligand binding to each of the receptor populations we studied have been previously reported (8–11, 15–17, 19–22), with one exception: the use of [^{3}H]prazosin and [^{3}H]yohimbine to label selectively the respective α_1- and α_2-receptor populations of rabbit uterus has been recently characterized by T. N. Lavin and R. J. Lefkowitz.[2]

The sources of the other reagents used include the following: dobutamine (Eli Lilly and Co., Indianapolis, Ind.), phentolamine (Ciba Pharmaceutical Co., Summit, N. J.), (−) epinephrine, (−) isoproterenol, and (±) propranolol (Sigma Chemical Co., St. Louis, Mo.).

Protein was determined by the method of Lowry et al. (23).

Adenylate cyclase assay. We prepared human platelet lysates and assayed adenylate cyclase activity as previously described by Tsai and Lefkowitz (24), with two exceptions: we utilized hypotonic lysis and mechanical homogenization for preparation of platelet lysates without freezing the membranes; and incubations were carried out for 10 min at 37°C. We assessed the intrinsic activities of (−) epinephrine, a compound known to be a full agonist at α_2-receptors (24), and of dobutamine by their respective abilities to inhibit the stimulation of adenylate cyclase elicited by 10 μM prostaglandin (PG)E_1. Triplicate determinations of adenylate cyclase activity were assessed at each drug concentration in three separate membrane preparations.

[1] *Abbreviations used in this paper*: DHA, [^{3}H]dihydroalprenolol; DHE, [^{3}H]dihydroergocryptine; GPP, guanyl-5′-yl imidodiphosphate; K_D, dissociation constant; PGE_1, prostaglandin E_1.

[2] Lavin, T. N., and R. J. Lefkowitz. Manuscript submitted for publication.

TABLE I

Experimental Conditions and Radioligand Binding Characteristics for each Membrane Preparation under Study

Membrane preparation	Concentration membrane	Radioligand	Concentration radioligand	Radioligand K_D	Total binding	Specific binding
	mg protein/ml		*nM*	*nM*	*pM**	*%*
Rat heart	1.8	DHA	4.0	1.9	53±1	69
	1.7	DHE	2.0	1.7	58±4	50
	1.7	[^{3}H]Prazosin	0.42	0.36	57±4	85
Frog heart	1.3	DHA	6.0	1.2	63±6	81
Turkey erythrocyte	1.6	DHA	10.5	5.9	328±21	87
Rat lung	0.35	DHA	3.7	2.0	164±34	71
Rabbit uterus	5.1	[^{3}H]Prazosin	1.3	0.50	129±39	71
	5.1	[^{3}H]Yohimbine	13.3	10.0	349±97	56
Human platelet	1.1	DHE	4.2	7.8	300±56	75

* Mean±SE.

Data analysis. In each experiment analyzing the dose-response relationship of dobutamine competition for DHA, DHE, [^{3}H]prazosin or [^{3}H]yohimbine binding sites in the membrane preparation under study, the binding of radioligand in the presence of each concentration of dobutamine was first normalized on a scale where binding in the presence of no added dobutamine was set at 100% and the binding in the presence of either 1 μM or 10 μM propranolol (β-receptors) or 10 μM phentolamine (α-receptors) was set at 0%. The normalized binding values at each concentration of dobutamine from separate experiments were meaned, and the pooled dose-response curves obtained in this fashion were utilized for all subsequent analyses.

Each competition curve was analyzed by a nonlinear least-squares curve-fitting procedure employing a generalized model for complex ligand-receptor interactions and performed using an iterative program in PL/1 on a PDP 11/45 computer. Fitted parameter estimates were thus obtained for each competition curve. The details of this analytical method have been previously described (25). To determine the significance between parameter estimates (such as K_D) from two separate competition curves, each curve was first analyzed independently determining the K_D estimates which provided the "best fit" of each separate dose-response curve. The curves were then analyzed simultaneously constraining the K_D estimate for dobutamine competition for radioligand binding in curve 1 to be equivalent to the K_D estimate for curve 2. The goodness of fit obtained by analyzing the data first independently, and then with this constraint, was assessed by measuring the residual variance between the data and each of the fitted curves, and comparing these variances by an F ratio test. For example, the K_D estimated from dobutamine competition for radioligand binding in one tissue homogenate (A) would be considered significantly different from the K_D estimated from dobutamine competition for radioligand binding in a different homogenate (B) when the residual variance of curves constraining K_D (A) = K_D (B) was increased significantly (by F test) from the residual variance observed when K_D (A) and K_D (B) were estimated independently.

RESULTS

We observed dobutamine to displace adrenergic radioligands from binding sites in each membrane preparation to an equivalent degree to the maximal inhibition produced by 10 μM or 1 μM propranolol for DHA binding or for 10 μM phentolamine for DHE, [^{3}H]prazosin, or [^{3}H]yohimbine binding. This indicates that dobutamine was indeed competing for the specific binding sites that represent physiologically relevant adrenergic receptors.

Beta adrenergic subtype specificity. Dobutamine exhibited greater affinity for beta adrenergic receptors of the β_1-than of the β_2-subtype. The dissociation constants for dobutamine in membranes derived from rat heart and turkey erythrocytes, which are predominantly of the β_1-subtype (8, 22), were significantly lower than those observed in membranes derived from frog heart and rat lung, which contain predominantly β_2-receptors (8, 10) (Table II; Figs. 1 and 2). In addition, dobutamine competition curves in rat cardiac membranes quantitated in the absence of guanine nucleotide were best characterized by two classes of binding sites: a high affinity site (K_D = 0.5 μM) representing 44% of the receptor population, and a lower affinity site (K_D = 5 μM) representing the remaining 56% of the receptors present. In the presence of 0.1 mM GPP, only a single class of binding sites of the lower affinity (K_D = 5.2 μM) was observed (Fig. 1). The formation of this high affinity state, which is reversible by guanine nucleotide, is characteristic of beta adrenergic agonists in rat heart (8). On the other hand, dobutamine competition curves to β_2-receptors of rat lung in the absence of guanine nucleotide were described best by only a single class of binding sites, and were shifted only minimally by 0.1 mM GPP. Shifts in agonist affinity for beta$_2$ receptors of rat lung induced by guanine nucleotides have been previously well characterized (13), and we observed similar effects of 0.1 mM GPP on (−) isoproterenol competition curves for DHA binding in our rat lung

TABLE II
Inhibition of Radioligand Binding to Adrenergic Receptor Subtypes by Dobutamine

Tissue	Radioligand	Subtype	Dobutamine K_D*	P‡
			μM	
Rat heart§ (4)	DHA	β_1	5.2±0.6	<0.001
Frog heart (3)	DHA	β_2	14.8±2.8	
Turkey erythrocyte (2)	DHA	β_1	2.6±0.4	<0.001
Rat lung (3)	DHA	β_2	25.4±3.1	
Rat heart (3)	DHE	α_1	0.17±0.05	
Rat heart (4)	[^{3}H]Prazosin	α_1	0.09±0.01	<0.001‖
Human platelet (2)	DHE	α_2	9.3±1.4	
Rabbit uterus (3)	[^{3}H]Prazosin	α_1	0.14±0.04	<0.001
Rabbit uterus (3)	[^{3}H]Yohimbine	α_2	5.7±1.4	

* Except for turkey erythrocyte DHA binding, rat heart DHE binding, and rat heart [^{3}H]prazosin binding, the values expressed here are derived from experiments performed in the presence of 0.1 mM guanyl-5′-yl imidodiphosphate (mean ±SE). The statistical comparisons were made on competition curves obtained in the presence of quanine nucleotide because such curves demonstrate only a single class of binding sites, and the derived parameter estimates are more readily compared than those derived from complex competition curves obtained in the absence of guanine nucleotide, which, in some cases, demonstrated two classes of binding sites (e.g., dobutamine competition for DHA binding in rat heart; Fig. 1).

‡ F ratio test. See text for description of statistical methods.

§ Numbers in parentheses refer to the number of experiments from which data are derived.

‖ Significance of rat heart (3) vs. human platelet and of rat heart (4) vs. platelet.

membrane preparation (Fig. 2). In a manner analogous to the observations in rat lung membranes, we observed in a single experiment no effect of 0.1 mM GPP on dobutamine competition curves for DHA binding in frog heart membranes (data not shown). Presumably because the preparation contains endogenous guanine nucleotide, the β_1-receptors of crude (as opposed to highly purified) turkey erythrocyte membranes do not exhibit this shift in agonist affinities with the addition of guanine nucleotides (26), and we did not test guanine nucleotide effects upon dobutamine binding in this tissue.

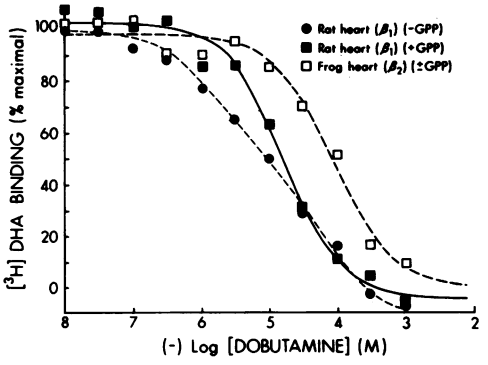

FIGURE 1 Dobutamine competition for DHA binding in rat heart and frog heart membranes. Each point is the mean of single or duplicate determinations in four (rat heart) or three (frog heart) separate experiments. 100% maximal binding refers to DHA binding in the absence of dobutamine and 0% binding refers to DHA binding to the presence of 10 μM (±) propranolol. GPP 0.1 mM had no effect on dobutamine displacement of DHA binding in frog heart, so the binding values for curves obtained in the presence and absence of GPP were pooled. The lines represent computer-derived "best fits" of the data points for each curve.

It should be noted that if one considers only the high affinity site for dobutamine binding to rat heart receptors observed in the absence of guanine nucleotides, the β_1-selectivity of dobutamine assessed by comparing its affinity for beta receptors of rat heart vs. rat lung or frog heart increases to >10-fold, from the 3-fold reported in Table II.

Since (−) isoproterenol binds equally well to both β_1- and β_2-receptors, we compared the relative affinities of dobutamine and (−) isoproterenol in competing for DHA binding in each of our beta receptor populations. The K_D for (−) isoproterenol in each tissue was either

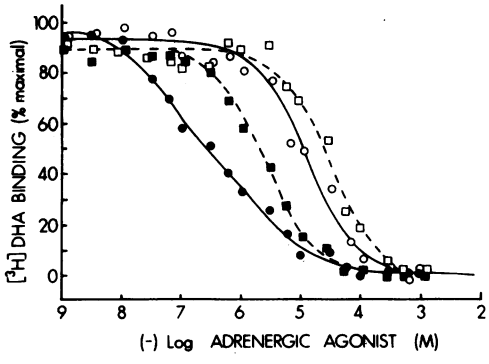

FIGURE 2 Dobutamine (open symbols) and (−) isoproterenol (filled symbols) competition for DHA binding in rat lung membranes in the presence (squares) and absence (circles) of 0.1 mM GPP. Each point is the mean of single determinations in three separate experiments. 100% maximal binding refers to DHA binding in the absence of dobutamine or (−) isoproterenol, and 0% binding refers to DHA binding in the presence of 1 μM (±) propranolol.

derived from published data (rat heart [8], frog heart [8], turkey erythrocyte [27]), or determined in separate experiments (rat lung). The ratio of K_D dobutamine to K_D (−) isoproterenol (in the presence of guanine nucleotide) in rat heart was 2.4 and was 2.8 in turkey erythrocyte, whereas this ratio was 7.1 in frog heart and 13.6 in rat lung.

Alpha adrenergic subtype selectivity. Dobutamine exhibited greater affinity for alpha adrenergic receptors of the α_1-subtype than of the α_2-subtype. The dissociation constant for dobutamine in membranes derived from rat heart, which are predominantly α_1 (19), was significantly lower than that observed in human platelet membranes, which are exclusively α_2 (20) (Table II, Fig. 3). Since rabbit uterine membranes contain a mixture of α_1- and α_2-receptors (20), we used the subtype-specific radioligand [^{3}H]prazosin and [^{3}H]yohimbine to identify selectively each respective receptor population. The dissociation constant derived from dobutamine competition for [^{3}H]prazosin binding to α_1-receptors was significantly lower than that observed for dobutamine competition for [^{3}H]yohimbine binding to α_2-receptors (Table II, Fig. 4).

Since guanine nucleotides influence agonist affinity at α_2-receptors in an analogous manner to the changes observed at β_1- and β_2-receptors (14), we studied the effects of 0.1 mM GPP on dobutamine and (−) epinephrine binding to α_2-receptors of human platelets. As seen in Fig. 5, (−) epinephrine competition for DHE binding in platelet membranes in the absence of GPP

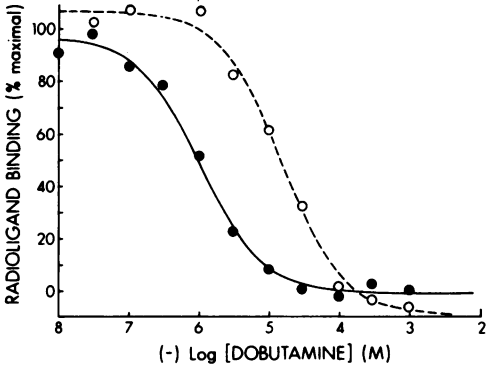

FIGURE 3 Dobutamine competition for [^{3}H]prazosin (●) binding in rat cardiac membranes and for DHE (○) binding in human platelet membranes. Each point is the mean of single or duplicate determinations in four (heart) or two (platelet) separate experiments. The platelet curve shown here demonstrates the binding in the presence of 0.1 mM GPP. Because guanine nucleotides do not alter agonist competition curves for [^{3}H]prazosin or DHE binding in rat cardiac membranes (see text), the data for rat heart binding shown here were obtained only in the absence of GPP. 100% maximal binding refers to radioligand binding in the absence of dobutamine, and 0% binding refers to binding in the presence of 10 μM phentolamine.

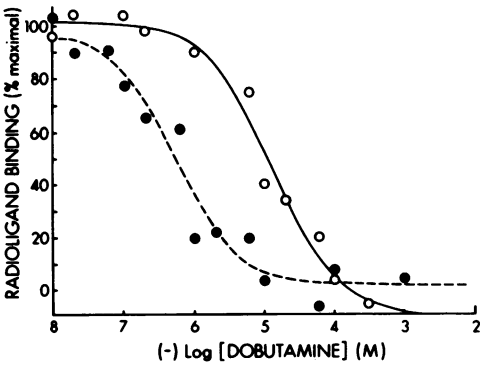

FIGURE 4 Dobutamine competition for [^{3}H]prazosin (●) and [^{3}H]yohimbine (○) binding in rabbit uterine membranes. Each point is the mean of single or duplicate determinations in three separate experiments. Both curves were obtained in the presence of 0.1 mM GPP. For an explanation of the units on the ordinate, see legend to Fig. 3.

is best characterized by the assumption of two classes of binding sites, of which the high affinity site is not observed in the presence of GPP. This pattern is typical for α_2-agonist compounds. On the other hand, guanine nucleotide had no effects on dobutamine competition for DHE binding sites.

These competitive binding data at α_2-receptors were extended by our analysis of the effects of (−) epinephrine and dobutamine upon PGE_1-stimulated adenylate cyclase activity in human platelet lysates. In three experiments, 10 μM PGE_1 increased adenylate cyclase activity 26.5-, 20.2-, and 24.0-fold over basal values. We observed a dose-dependent inhibition of PGE_1-stimulated adenylate cyclase activity by (−) epinephrine (which could be blocked by 10 μM phentolamine, but not by 10 μM [±] propranolol), whereas dobutamine

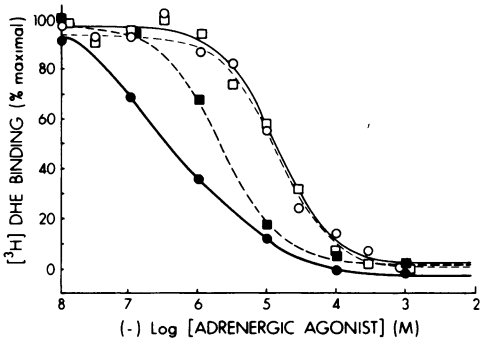

FIGURE 5 Dobutamine (open symbols) and (−) epinephrine (filled symbols) competition for DHE binding to human platelet membranes in the presence (squares) and absence (circles) of guanine nucleotide (0.1 mM GPP). Each point represents the mean of duplicate determinations in either five (epinephrine) or two (dobutamine) separate experiments. For an explanation of the units on the ordinate, see legend to Fig. 3.

lacked this effect (Table III). The EC_{50} for (−) epinephrine inhibition of PGE_1-stimulated adenylate cyclase activity was 1.9 μM, paralleling its potency in the competitive binding assay (Fig. 5).

DISCUSSION

The widely accepted view (28) that both chronotropic and inotropic responses to catecholamines are mediated exclusively through cardiac adrenergic receptors of the β_1-subtype has recently been challenged on two fronts. Persuasive evidence supporting the existence of functional postsynaptic cardiac alpha adrenergic receptors influencing both chronotropy and inotropy has been presented (15, 29–31); and some investigators have advanced the hypothesis that cardiac receptors of the β_2-subtype may play a role in mediating chronotropic, but not inotropic, responses to catecholamines. For example, Carlsson et al. (32) observed in a reserpinized anesthetized cat model that the ratio of the drug dose required to produce a 50% maximal chronotropic response to the dose required to produce a 50% maximal inotropic response was 0.93 for the β_1-selective agonist (−) H80/60, 0.5 for the non-subtype selective agonist (−) isoproterenol, and 0.16 for the β_2-selective agonist terbutaline. Other authors have reported proportionately greater effects upon heart rate than upon contractility from β_2-selective, as opposed to non-subtype selective or β_1-selective agents in other experimental preparations (33–37).

TABLE III
Inhibition of Prostaglandin-stimulated Adenylate Cyclase Activity in Human Platelets

Drug concentration	Adenylate cyclase activity	
	(−) Epinephrine	Dobutamine
	% PGE_1 response	
0	100	100
0.1 μM	99±3*	108±1‡
1 μM	76±10	117±4
3 μM	63±14	120±4
10 μM	51±10	118±4
30 μM	43±12	122±7
0.1 mM	41±7	116±10
0.3 mM	38±8	116±14
1 mM	34±6	84±19

* Mean±SD of triplicate determinations from three experiments.

‡ The increases in adenylate cyclase activity above that induced by PGE_1, which we observed in the presence of dobutamine, may reflect beta adrenergic agonist effects of dobutamine that are unopposed by α_2-agonist effects. These small increases were blunted by 10 μM (±) propranolol. Propranolol had no effect on the inhibition of PGE_1-stimulated adenylate cyclase activity induced by (−) epinephrine, whereas this response was abolished by 10 μM phentolamine.

The relative lack of positive chronotropic effects of dobutamine compared with those produced by isoproterenol at doses of each compound eliciting equivalent positive inotropic effects has been demonstrated in clinical studies (2, 38, 39), in both conscious and anesthetized dog models (1–4) and in some isolated heart preparations (1, 40). Although other investigators have failed to observe an inotropic selectivity in isolated myocardial preparations (41, 42), there is agreement that the inotropic selectivity of dobutamine in vivo is not fully attributable to baroreceptor influences, or to effects on endogenous norepinephrine release, degradation, or reuptake (1–3).

By what mechanism, then, are the pharmacologic responses to dobutamine mediated? The relative selectivity of dobutamine for inotropic as opposed to chronotropic stimulation, when compared with (−) isoproterenol, renders untenable the hypothesis that both agents exert their direct cardiac effects exclusively through identical β_1-receptors. An alternative hypothesis is that dobutamine is relatively selective for stimulation of β_1- as opposed to β_2-adrenergic receptors, and that the chronotropic responses to catecholamines are indeed mediated more by agonist binding to β_2 than to β_1-receptors. Our current data demonstrate a greater affinity of dobutamine for β_1- than for β_2-receptors in vitro, and, by virtue of the minimal ability of dobutamine to form the high affinity agonist-receptor complex which is reversed by guanine nucleotide at β_2-receptors in vitro, predict that dobutamine should have minimal stimulatory effects upon physiologic or biochemical responses mediated through β_2-receptors. Previous pharmacologic data confirm this prediction: dobutamine has only minimal effects upon peripheral β_2-receptors mediating relaxation of vascular smooth muscle (1–4), and previous studies of biochemical responses to dobutamine have also suggested that this compound has little agonist activity at β_2-receptors. For example, Pike and Lefkowitz (43) found dobutamine to be a partial agonist for stimulation of adenylate cyclase or of GTPase activity in turkey erythrocyte membranes (β_1) with peak stimulatory activity 24 and 19%, respectively, of that observed for (−) isoproterenol, whereas dobutamine had only minimal activity for stimulation of adenylate cyclase (8% of [−] isoproterenol response) or for GTPase activity (<5% of [−] isoproterenol response) in frog erythrocyte membranes (β_2).

The surprisingly high affinity (greater than [−] epinephrine) of dobutamine that we observed for $alpha_1$ adrenergic receptors in rat cardiac and rabbit uterus membrane homogenates suggests an alternative hypothesis regarding the inotropic vs. chronotropic selectivity of dobutamine's action. Since stimulation of cardiac α_1-receptors has been reported to augment inotropy while reducing the rate of phase 4 depolarization (reduce chronotropy) in cardiac tissue (29–31),

perhaps direct α_1-agonist activity in the heart could account for dobutamine's distinctive effects. The work of Tuttle and Mills (1) suggested that dobutamine's inotropic effects were only minimally affected by phenoxybenzamine, but the effects of alpha blockade on the heart rate responses to dobutamine have not, to our knowledge, been reported. Since agonist binding to α_1-receptors does not appear to be markedly influenced by guanine nucleotides in rat heart[3] and rabbit uterus (14), we cannot distinguish α_1-agonists from α_1-antagonists by radioligand binding experiments alone, and further studies will be required to resolve the agonist-antagonist nature of the high affinity α_1-binding of dobutamine that we observed.

Because previous pharmacologic investigations of dobutamine action have suggested that dobutamine functions neither as an alpha adrenergic agonist nor as an antagonist in vascular smooth muscle, some further consideration of the functional significance of dobutamine's high affinity α_1-binding is required. An interesting hypothesis that would reconcile this apparent discrepancy has recently been formulated and supported by data from Langer (44) and Yamaguchi and Kopin (45). Studying blood pressure responses in pithed rats, these latter investigators have noted that the α_1 selective antagonists phenoxybenzamine and dibenamine were considerably more potent in blocking the pressor effects of direct sympathetic stimulation than in blocking the effects of infused exogenous norepinephrine. The converse was true for the α_2-antagonists mianserin and piperoxan. Citing the evidence for postsynaptic receptors of the α_2-subtype in other tissues (7), they proposed that two subpopulations of postsynaptic alpha adrenergic receptors mediating vasoconstriction are present in peripheral blood vessels: α_1-receptors localized to the region of the vascular neuro-effector junctions, and separate α_2-receptors which are the most important sites of action of infused or humorally circulating alpha agonists. If this hypothesis is valid, and if such a distinction between "intrasynaptic" α_1 and "extrasynaptic" (44) α_2 postsynaptic receptors of vascular smooth muscle is physiologically relevant, the relatively weak effects of infused dobutamine on vascular smooth muscle in vivo (1–4) would be predicted from our radioligand binding studies, which demonstrated a much greater affinity of dobutamine for α_1- as opposed to α_2-receptors, regardless of whether dobutamine is an α_1-agonist or antagonist.

Since dobutamine clearly binds to both β_2- and α_2-receptors, yet exhibits only weak agonist effects, it would be expected, at appropriate concentrations, to function as an antagonist at receptors of these subtypes. Dobutamine's binding affinities for β_2-receptors (K_D = 15–25 μM) and for α_2 receptors (K_D = 6–9 μM) in membrane preparations are considerably less than the affinities of more typical adrenergic antagonists such as propranolol (K_D = 0.0046 μM in frog erythrocyte [β_2] membranes [46]), or phentolamine or yohimbine (K_D = 0.005 and 0.0008 μM, respectively, in human platelet [α_2] membranes [20]). Nonetheless, it is plausible that antagonism of endogenous catecholamine effects mediated through β_2- or α_2-receptors may influence the pharmacologic actions of dobutamine. Furthermore, β_2- or α_2-antagonist activity by dobutamine could alter the effects of β_2- or α_2-agonist drugs that might be utilized concurrently with dobutamine. These possibilities have not, to our knowledge, been rigorously tested in animal preparations or in man, and seem to merit further study.

In a previous report, Minneman et al. (47) have assessed the affinity of dobutamine for inhibition of [^{125}I]iodohydroxybenzylpindolol binding to β_1-receptors of rat heart homogenates and to β_2-receptors of rat lung homogenates; they concluded that no subtype selectivity of dobutamine was present. The explanation for the discrepancy between those findings and our results is not readily evident; however, the more highly detailed nature of our current competition curves, coupled with the prior physiologic and biochemical response data suggesting that dobutamine has only weak activity at β_2-receptors, support the conclusion that dobutamine is indeed a β_1-selective ligand.

We conclude that dobutamine has greater affinity for β_1- than for β_2-adrenergic receptors, and for α_1- than for α_2-adrenergic receptors. In addition, analysis of guanine nucleotide effects on competitive binding assays for dobutamine would predict that dobutamine has agonist properties at β_1-receptors, but little or no agonist activity at β_2- or α_2-receptors, and we have confirmed this conclusion directly for α_2 receptors. The physiologic importance of findings obtained in studies of cell-free systems must always be interpreted with caution and, as other authors have suggested (41, 42), dobutamine's inotropic selectivity may be unrelated to its receptor binding characteristics. However, our data regarding the adrenergic receptor subtype selectivity of dobutamine binding in vitro are best reconciled to its known pharmacologic properties by the two recent hypotheses suggesting a functional heterogeneity of (*a*) the subtypes of beta adrenergic receptors mediating chronotropy as opposed to inotropy in the myocardium, and (*b*) the subtypes of postsynaptic alpha adrenergic receptors mediating vasoconstriction of vascular smooth muscle in response to exogenously administered, as opposed to endogenously released, adrenergic agonists.

[3] Williams, R. S., and R. J. Lefkowitz. Manuscript in preparation.

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REFERENCES

1. Tuttle, R. R., and J. Mills. 1975. Dobutamine: development of a new catecholamine to selectively increase cardiac contractility. *Circ. Res.* **36:** 185–196.
2. Sonnenblick, E. H., W. H. Frishman, and T. H. LeJemtel. 1979. Dobutamine: a new synthetic cardioactive sympathetic amine. *N. Engl. J. Med.* **300:** 17–22.
3. Robie, N. W., D. D. Nutter, C. Moody, and J. L. McNay. 1974. In vivo analysis of adrenergic receptor activity of dobutamine. *Circ. Res.* **34:** 663–671.
4. Vatner, S. F., R. J. McRitchie, and E. Braunwald. 1974. Effects of dobutamine on left ventricular performance, coronary dynamics, and distribution of cardiac output in conscious dogs. *J. Clin. Invest.* **53:** 1265–1273.
5. Ahlquist, R. P. 1948. A study of the adrenotropic receptors. *Am. J. Physiol.* **153:** 586–600.
6. Lands, A. M., A. Arnold, J. P. McAuliff, F. P. Ludena, and T. G. Brown. 1967. Differentiation of receptor systems by sympathomimetic amines. *Nature (Lond.).* **214:** 597–598.
7. Berthelsen, S., and W. A. Pettinger. 1977. A functional basis for classification of α-adrenergic receptors. *Life Sci.* **21:** 595–606.
8. Hancock, A. A., A. L. DeLean, and R. J. Lefkowitz. 1979. Quantitative resolution of beta-adrenergic receptor subtypes by selective ligand binding: application of a computerized model fitting program. *Mol. Pharmacol.* **16:** 1–9.
9. Hoffman, B. B., and R. J. Lefkowitz. 1980. Alpha-adrenergic receptor subtypes. *N. Engl. J. Med.* **302:** 1390–1396.
10. U'Prichard, D. C., D. B. Bylund, and S. H. Synder. 1978. (±) [^{3}H]Epinephrine and (−) [^{3}H]dihydroalprenolol binding to β_1- and β_2-noradrenergic receptors in brain, heart, and lung membranes. *J. Biol. Chem.* **253:** 5090–5102.
11. Minneman, K. P., A. Hedberg, and P. B. Molinoff. 1979. Comparison of beta adrenergic receptor subtypes in mammalian tissues. *J. Pharmacol. Exp. Ther.* **211:** 502–508.
12. Lefkowitz, R. J., D. Mullikin, and M. C. Caron. 1976. Regulation of β-adrenergic receptors by guanyl-5′-yl imidodiphosphate and other purine nucleotides. *J. Biol. Chem.* **251:** 4686–4692.
13. Hegstrand, L. R., K. P. Minneman, and P. B. Molinoff. 1979. Multiple effects of guanosine triphosphate on beta adrenergic receptors and adenylate cyclase activity in rat heart, lung and brain. *J. Pharmacol. Exp. Ther.* **210:** 215–221.
14. Hoffman, B. B., D. Mullikin-Kilpatrick, and R. J. Lefkowitz. 1980. Heterogeneity of radioligand binding to α-adrenergic receptors: analysis of guanine nucleotide regulation of agonist binding in relation to receptor subtypes. *J. Biol. Chem.* **255:** 4645–4652.
15. Williams, R. S., and R. J. Lefkowitz. 1978. Alpha-adrenergic receptors in rat myocardium: identification by binding of [^{3}H]dihydroergocryptine. *Circ. Res.* **43:** 721–727.
16. Newman, K., L. T. Williams, N. Bishopric, and R. J. Lefkowitz. 1978. Identification of platelet alpha-adrenergic receptors by [^{3}H]dihydroergocryptine binding. *J. Clin. Invest.* **61:** 395–402.
17. Williams, L. T., D. Mullikin, and R. J. Lefkowitz. 1976. Identification of α-adrenergic receptors in uterine smooth muscle membranes by [^{3}H]dihydroergocryptine binding. *J. Biol. Chem.* **251:** 6915–6923.
18. Hoffman, B. B., D. Mullikin-Kilpatrick, and R. J. Lefkowitz. 1979. Desensitization of beta-adrenergic stimulated adenylate cyclase in turkey erythrocytes. *J. Cyclic Nucleotide Res.* **5:** 355–366.
19. Williams, R. S., D. F. Dukes, and R. J. Lefkowitz. 1981. Subtype specificity of alpha adrenergic receptors in rat heart. *J. Cardiovasc. Pharmacol.* In press.
20. Hoffman, B. B., A. L. DeLean, C. L. Wood, D. D. Schocken, and R. J. Lefkowitz. 1979. Alpha-adrenergic receptor subtypes: quantitative assessment by ligand binding. *Life Sci.* **24:** 1739–1746.
21. Wood, C. L., C. D. Arnett, W. R. Clarke, B. S. Tsai, and R. J. Lefkowitz. 1979. Subclassification of alpha-adrenergic receptors by direct binding studies. *Biochem. Pharmacol.* **28:** 1277–1282.
22. Atlas D., M. L. Steer, and A. Levitzki. 1974. Stereospecific binding of propranolol and catecholamines to the β-adrenergic receptor. *Proc. Natl. Acad. Sci. U. S. A.* **71:** 4246–4248.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
24. Tsai, B. S., and R. J. Lefkowitz. 1979. Agonist-specific effects of guanine nucleotides on alpha-adrenergic receptors in human platelets. *Mol. Pharmacol.* **16:** 61–68.
25. Kent, R. S., A. DeLean, and R. J. Lefkowitz. 1980. A quantitative analysis of beta-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. *Mol. Pharmacol.* **17:** 14–23.
26. Brown, E. M., S. A. Fedak, C. J. Woodward, G. D. Aurbach, and D. Rodbard. 1976. β-adrenergic receptor interactions: direct comparison of receptor interaction and biological activity. *J. Biol. Chem.* **251:** 1239–1246.
27. Stadel, J. M., A. DeLean, and R. J. Lefkowitz. 1980. A high affinity agonist β-adrenergic receptor complex is an intermediate for catecholamine stimulation of adenylate cyclase in turkey and frog erythrocyte membranes. *J. Biol. Chem.* **255:** 1436–1441.
28. Koelle, G. B. 1975. Neurohumoral transmission and the autonomic nervous system. *In* The Pharmacologic Basis for Therapeutics. L. S. Goodman and A. Gilman, editors. McMillan Publishing Co., New York. 404–444.
29. Govier, W. C., N. C. Mosal, P. Whittington, and A. H. Broom. 1966. Myocardial alpha and beta-adrenergic receptors as demonstrated by atrial function refractory period changes. *J. Pharmacol. Exp. Ther.* **154:** 255–263.
30. Mary-Rabine, L., A. J. Hordof, F. O. Bowman, J. R. Malm, and M. R. Rosen. 1978. Alpha and beta-adrenergic effects on human atrial specialized conducting fibers. *Circulation.* **57:** 84–90.
31. Rabinowitz, B., L. Chuck, M. Kligerman, and W. W. Parmley. 1975. Positive inotropic effects of methoxamine: evidence for alpha-adrenergic receptors in ventricular myocardium. *Am. J. Physiol.* **229:** 582–585.
32. Carlsson, E., C. Dahlof, A. Hedberg, H. Persson and B. Tangstrand. 1977. Differentiation of cardiac chronotropic and inotropic effects of β-adrenergic agonists. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **300:** 101–105.
33. Carlsson, E., B. Ablad, A. Brandstrom, and B. Carlsson. 1972. Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart. *Life Sci.* **11**(I): 953–958.
34. Furnival, C. M., R. J. Linden, and H. M. Snow. 1971. The inotropic and chronotropic effects of catecholamines on the dog heart. *J. Physiol. (Lond.).* **214:** 15–28.

35. Brittain, R. T., D. Jack, and A. C. Ritchie. 1970. Recent β-adrenergic stimulants. *Adv. Drug Res.* **5**: 197–253.
36. Farmer, J. B., G. P. Levy, and R. J. Marshall. 1970. A comparison of the β-adrenergic stimulant properties of salbutamol, orciprenaline and soternol with those of isoprenaline. *J. Pharm. Pharmacol.* **22**: 945–947.
37. Ablad, B., K. O. Borg, E. Carlsson, L. Ek, G. Johnsson, T. Malmfors, and C. Regardh. 1975. A survey of the pharmacological properties of metoprolol in animals and man. *Acta Pharmacol. Toxicol.* **36**(Suppl. V): 7–23.
38. Liers, C. V., P. T. Heban, P. Huss, C. A. Bush, and R. P. Lewis. 1978. Comparative systemic and regional hemodynamic effects of dopamine and dobutamine in patients with cardiomyopathic heart failure. *Circulation.* **58**: 466–475.
39. Stephens, J., H. Ead, and R. Spurrell. 1979. Hemodynamic effects of dobutamine with special reference to myocardial blood flow: a comparison with dopamine and isoprenaline. *Br. Heart J.* **42**: 43–50.
40. Tuttle, R. R. C. C. Hillman, and R. E. Toomey. 1976. Differential β-adrenergic sensitivity of atrial and ventricular tissue assessed by chronotropic, inotropic, and cyclic AMP responses to isoprenaline and dobutamine. *Cardiovasc. Res.* **10**: 452–458.
41. Bodem, R., C. L. Skeleton, and E. H. Sonnenblick. 1974. Inotropic and chronotropic effects of dobutamine on isolated cardiac muscle. *Eur. J. Cardiol.* **2**: 181–189.
42. Lumley, P., K. J. Broadley, and G. P. Levy. 1977. Analysis of the inotropic: chronotropic selectivity of dobutamine and dopamine in anesthetized dogs and guinea pig isolated atria. *Cardiovasc. Res.* **11**: 17–25.
43. Pike, L. J., and R. J. Lefkowitz. 1980. Activation and desensitization of β-adrenergic receptor-coupled GTPase and adenylate cyclase of frog and turkey erythrocyte membranes. *J. Biol. Chem.* **255**: 6860–6867.
44. Langer, S. Z. 1980. Subclassification of α-adrenoceptors into α_1 and α_2 types: relevance to cardiovascular effects of drugs. *Circulation.* **62**(Suppl. III): 6–7.
45. Yamaguchi, I., and I. J. Kopin. 1980. Differential inhibition of α_1- and α_2-adrenoceptor-mediated pressor responses in pithed rats. *J. Pharmacol. Exp. Ther.* In press.
46. Mukherjee, C., M. G. Caron, M. Coverstone, and R. J. Lefkowitz. 1974. Identification of adenylate-cyclase coupled beta-adrenergic receptors in frog erythrocytes with (−) [^{3}H]alprenolol. *J. Biol. Chem.* **250**: 4869–4876.
47. Minneman, K. P., L. R. Hegstrand, and P. B. Molinoff. 1979. The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung in vitro. *Mol. Pharmacol.* **16**: 21–33.