Effect of GTP on the Affinity of Denopamine, a New Cardiotonic Agent, for β -Adrenergic Receptors of Turkey Erythrocytes and Rat Reticulocyte Membranes

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Abstract—Affinities of denopamine, a new cardiotonic agent, and several β -adrenergic drugs for turkey erythrocyte membranes (TEM) and rat reticulocyte membranes (RRM) which contain homogeneous β_1 - and β_2 -receptors, respectively, were studied by receptor binding. The order of potencies of denopamine and several β -adrenergic agonists in displacing ^3H -dihydroalprenolol binding (K_i , nM) in TEM was isoproterenol (Iso, 27) > norepinephrine (Nor, 360) > epinephrine (Epi, 860) > dobutamine (DB, 1380) > denopamine (1540) > dopamine (DA, 49500). The order in RRM was Iso (7.3) > Epi (58) > DB (750) > Nor (1090) > denopamine (2300) > DA (26800). In the presence of GTP, competition curves for full agonists like Iso, Epi and Nor shifted to the low affinity side (K_i values increased by 300–500% in TEM and 200–460% in RRM), and the slopes were steepened in both membrane preparations. The K_i value for denopamine increased only in TEM (70%) and that in RRM was not influenced by GTP. This suggests that denopamine has an agonist property at the β_1 -receptor but not at the β_2 -receptor and that the intrinsic activity at the β_1 -receptor of the drug is lower than full agonists. Affinities of DB and DA for TEM were influenced by GTP as well as those for RRM, although the extent of the rightward shift was less than full agonists.

Denopamine, (–)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxy-phenethyl)amino] ethanol, is a new, orally active, positive inotropic agent with a weak chronotropic action (1–3). In the previous paper, we reported that denopamine exhibited a β_1 -selective affinity and agonist property (4) by radioligand binding (5–7), using rat heart membranes and lung membranes. In the above report, we demonstrated that the degree of the rightward shift of the competition curve for denopamine by the addition of GTP was less than those for full agonists like isoproterenol, epinephrine and norepinephrine. Since it has been recognized that the degrees of the GTP-induced rightward shift well correspond to the intrinsic activities of β -agonists (8, 9), the agonist property of denopamine was considered to be weak as compared with full agonists. However, the effect of GTP on the affinities

for β -receptors in the previous experiment may be obscured by the coexistence of β_1 - and β_2 -receptor subtypes in both membrane preparations. The present experiment was carried out to further investigate the agonist property of this drug to the β_1 - and β_2 -receptors using turkey erythrocyte membranes and rat reticulocyte membranes which were reported to contain homogeneous β_1 - and β_2 -receptors, respectively (10–13). In addition, we compared the degrees of the rightward shift by GTP among several β -adrenergic agonists in these membrane preparations.

Materials and Methods

1. Preparation of turkey erythrocyte membranes and rat reticulocyte membranes: The turkey erythrocyte membranes (TEM) and rat reticulocyte membranes (RRM) were prepared by the method of Charness et al.

(14) with some modifications. Blood was sampled from the axillary vein of male turkeys (about 12 months of age). Reticulocyte-rich blood was obtained from the abdominal aorta of male Sprague-Dawley rats (6–7 weeks of age) injected intraperitoneally with a solution of phenylhydrazine hydrochloride (adjusted to pH 7.0 with saturated aqueous solution of NaHCO_3) in a dose of 50 mg/kg/day for 3 days. Rats were sacrificed on the 7th day after the first administration of phenylhydrazine hydrochloride.

One ml of 0.2 M sodium EDTA was added to the blood sample (10 ml) as an anti-coagulant. After blood was centrifuged at $2000\times g$ for 5 min, the plasma and buffy coat were removed by aspiration. The cells were two times washed with two volumes of cold 150 mM NaCl, 5 mM Tris-HCl buffer (pH 7.5), followed by centrifugation. Using a syringe with polyethylene tubing, 3 ml (in the case of TEM) or 1 ml (RRM) of packed cells were sampled from the bottom of the tube and lysed in 40 volumes of 5 mM Tris-HCl buffer (pH 7.5) for 20 min with stirring. After the addition of 3 ml (TEM) or 1 ml (RRM) of the mixtures of 4 M KCl and 40 mM MgCl_2 , the lysate was centrifuged at $28000\times g$ for 10 min. The pellet was resuspended, above lysis step was repeated, and the membrane pellet was finally suspended in 100 ml of 50 mM Tris-HCl buffer (pH 7.5, 25°C). Erythrocyte membranes from nontreated rats were prepared in the same way as RRM. In all the experiments, the final protein concentrations of TEM, RRM and erythrocyte membranes were about 300, 50 and 50 $\mu\text{g}/\text{ml}$, respectively. In TEM, 10 mM MgCl_2 and 1 mM EDTA were added to the membrane suspension. The above operations were conducted at 2°C, and all experiments were carried-out on the day of preparation.

2. ^3H -Dihydroalprenolol (DHA) binding assays: The ^3H -DHA binding experiment was carried out by the same method reported previously (4). For the binding saturation curves, an aliquot (1 ml) of the rat erythrocyte or reticulocyte membrane suspension was incubated for 30 min at 25°C with various concentrations of ^3H -DHA (about 0.1–5 nM) in a total volume of 1.1 ml. In the case of TEM, incubation was conducted at 37°C.

Reaction was stopped by the addition of 8 ml of the ice-cold 50 mM Tris-HCl buffer. The incubated mixture was rapidly filtered through glass fiber filters (Whatman, GF/C) under a vacuum. Each filter was washed with an additional 8 ml of ice-cold buffer. Membrane-bound radioactivity on the filter was determined in a toluene-Triton X-100 scintillation cocktail (12 g DPO, 300 mg POPOP, 2 l toluene, 1 l Triton X-100, 300 ml methanol) using a liquid scintillation spectrometer (Packard, 460 CD). Specific binding of ^3H -DHA was defined as the amount of ^3H -DHA bound in the absence of a competing drug minus the amount bound in the presence of 10 μM ($\pm$)-propranolol. Specific binding of ^3H -DHA to TEM and RRM was 70–98% of the total ^3H -DHA binding, respectively.

In the binding inhibition studies, an aliquot (1 ml) of the membrane suspension was incubated with ^3H -DHA (about 0.78 nM in TEM and 0.2 nM in RRM) and various concentrations of competing drugs. Specific binding was obtained as described above. Ascorbic acid (0.1 mM) and pargyline (1 μM) were included in the incubation mixture to prevent oxidation of catecholamines. If necessary, a small amount of HCl (0.15 mM) was also added to facilitate solubilization of the test drugs. These chemicals were shown to have no effect on specific ^3H -DHA binding at the concentration used.

3. Determination of protein concentration: Protein concentration in the membrane fractions of rat erythrocytes and reticulocytes and turkey erythrocytes was determined by the method of Lowry et al. (15) using bovine serum albumin as a standard.

4. Analysis of data: The IC_{50} of radioligand competition curve was determined by the X-intercept of linearized plots of $\log [B/B_{\text{max}} - B]$ vs. $\log C$, where B is the amount of ^3H -DHA bound in the presence of competing drugs, B_{max} is the amount of the radioligand bound in the absence of competing drugs and C is the concentration of competing drugs. The slope (b, Hill coefficient) of the competition curve was derived from a logistic curve of 4 parameters described below (16):

$$Y = \frac{a - d}{1 + (X/c)^b} + d$$

where Y is a specific binding at the test drug concentration of X. Symbols of c, a and d are IC50, maximum (100%) and minimum (0%) value of the ^3H -DHA binding, respectively. Values of the inhibition constant (K_i) for the competing drugs were calculated from the IC50 by the following equation (17):

$$K_i = \text{IC}_{50} / (1 + L/K_d)$$

where L is the concentration of the radio-ligand in the incubation mixture, and K_d is the equilibrium dissociation constant of ^3H -DHA for the binding site. Student's *t*-test was employed to assess the statistical significance.

5. Materials: ^3H -Dihydroalprenolol (DHA, specific activity: 77 Ci/mmol) was obtained from Amersham. (–)-Isoproterenol D-bitartrate, (–)-norepinephrine, (–)-dopamine hydrochloride and (±)-propranolol hydrochloride were obtained from Nakarai Chemicals (Japan), and (–)-epinephrine was purchased from Merck. Denopamine and practolol were synthesized at the Organic Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd. (Toda, Saitama, Japan). Dobutamine hydrochloride, prenalterol hydrochloride and procaterol were extracted from the commercially available drugs. Other drugs and chemicals used in the experiment were as follows: guanosine 5'-triphosphate 3-Na (GTP, Yamasa Shoyu Co., Ltd., Japan), *L*-ascorbic acid, pargyline hydrochloride, phenylhydrazine hydrochloride, EDTA 2-Na and bovine serum albumin (Nakarai Chemicals).

Results

1. Characteristics of specific ^3H -DHA

binding to turkey erythrocyte membranes rat erythrocyte membranes and rat reticulocyte membranes: Table 1 shows the parameters for specific ^3H -DHA binding to turkey erythrocyte membranes (TEM), rat reticulocyte membranes (RRM) and rat erythrocyte membranes. The maturation of rat reticulocytes to erythrocytes resulted in a marked (about 3-fold) loss of B_{max} without significant changes in K_d value, showing agreement with previous papers (13, 18). In each membrane preparation, Scatchard plots (19) were linear and Hill coefficients (20) were nearly 1.0, indicating that ^3H -DHA bound to these membranes with a single population of binding sites.

^3H -DHA binding to RRM was rapid and saturable, reaching equilibrium by 10 to 20 min and remaining constant until 1 hr. By the addition of a large excess of propranolol (10^{-5} M), the specific binding was rapidly dissociated with a half-life of 10.2 min. By the method of Bylund and Snyder (21), the observed initial rate constant (k_{obs}), the association rate constant (k_1) and the rate constant for dissociation of ^3H -DHA binding (k_{-1}) to RRM were calculated to 0.173 min^{-1} , 0.482 $\text{nM}^{-1} \cdot \text{min}^{-1}$ and 0.068 min^{-1} , respectively. Therefore, the equilibrium dissociation constant (k_{-1}/k_1) for ^3H -DHA binding to RRM was estimated to be 0.141 nM, which practically agreed with the K_d value (0.103, Table 1) from Scatchard analysis.

Figure 1 shows Hofstee plots (7) for the inhibition of ^3H -DHA binding by the β_2 -selective agonist procaterol and the β_1 -

Table 1. Equilibrium binding parameters for ^3H -DHA to the rat erythrocyte and reticulocyte membranes and the turkey erythrocyte membranes

	Rat		Turkey
	Erythrocytes	Reticulocytes	Erythrocytes
K_d (nM)	0.127±0.016	0.103±0.008	0.778±0.020
B_{max} (pmol/mg prot.)	0.47 ±0.02	1.29 ±0.04*	0.31 ±0.02
R (Scatchard plot)	0.973±0.022	0.991±0.005	0.980±0.007
Hill coefficient	1.003±0.077	1.043±0.058	1.087±0.004
R (Hill plot)	0.983±0.005	0.976±0.019	0.990±0.002

Each value represents the mean and S.E. of 4 experiments, each performed in duplicate determinations. "R" represents the correlative coefficient for Scatchard or Hill plots. * $P < 0.01$, compared with rat erythrocyte membranes.

Fig. 1. Hofstee plots for the inhibition of ^3H -DHA binding by the β_2 -selective agonist procaterol and β_1 -selective antagonist practolol in rat reticulocyte membranes. Each point represents the mean of 4 experiments, each performed in triplicate determinations. ^3H -DHA binding was determined in the presence of 0.1 mM GTP. B is a % inhibition of specific ^3H -DHA binding and S is the concentration of competing drug (nM).

selective antagonist practolol in RRM. Computer analysis has shown that the population of β_2 -receptors in RRM was calculated to be $99.5 \pm 0.5\%$ (mean $\pm$ S.E., $N=4$, procaterol) and $96.1 \pm 2.2\%$ (practolol), indicating nearly homogeneous β_2 -receptors.

2. Effect of β -adrenergic drugs on ^3H -DHA binding to the turkey erythrocyte membranes (β_1): Figure 2(A) and Table 2 show the displacement curves, inhibition constants (K_i values) and Hill coefficients of β -adrenergic drugs for ^3H -DHA binding to TEM. For typical catecholamines, the potency of the inhibition of ^3H -DHA binding was in the order of isoproterenol (Iso, 26.5 nM) > norepinephrine (Nor, 360 nM) > epinephrine (Epi, 860 nM). This rank order was considered to reflect essentially the pharmacological characteristics of β_1 -adrenoceptor subtype (22). The potency of the binding inhibition of other drugs in TEM was in the order of prenalterol > dobutamine $\geq$ denopamine > procaterol > dopamine. The K_i value for denopamine (1540 nM) was compatible with that of dobutamine (1380 nM) and larger than that of prenalterol (560 nM). The affinity of dopamine was extremely low, and its K_i value was about 1/1900 that of Iso.

3. Effects of β -adrenergic drugs on ^3H -DHA binding to the rat reticulocyte membranes (β_2): Displacement curves and K_i values for β -adrenergic drugs in RRM are shown in Fig. 3(A) and Table 3. The potency of the inhibition of the ^3H -DHA binding was in the order of Iso (7.3 nM) > Epi (58 nM) >

Nor (1090 nM), indicating that RRM has a characteristic of the β_2 -subtype (22). In other drugs, the potency of the ^3H -DHA binding inhibition in RRM was in the order of procaterol > prenalterol > dobutamine > denopamine (2300 nM) > dopamine. These results show that the affinity of denopamine for the β_2 -receptor was comparably low. Practolol, a β_1 -antagonist, showed a very low affinity among the drugs tested.

4. Effects of GTP on the inhibition of ^3H -DHA binding to the turkey erythrocyte membranes and rat reticulocyte membranes: When 0.1 mM GTP was added to the incubation mixture, as shown in Tables 2 and 3 and Figs. 2(B) and 3(B), K_i values for three typical catecholamines, Iso, Epi and Nor, in TEM and RRM increased by 300–500% and 200–460%, respectively. Thus, it was demonstrated that the affinities of these drugs for both β_1 - and β_2 -receptors decreased by the addition of GTP. Especially, K_i values for Iso increased by 500% in TEM and about 460% in RRM. Denopamine showed less increase in K_i value in TEM (70%) than that for full agonists. In RRM, no significant changes in the inhibition curve for denopamine were observed like prenalterol, a β_1 -partial agonist, and practolol, a β_1 -antagonist. Whereas Hill coefficients of the competition curves for full agonists increased by the addition of GTP, those for denopamine and prenalterol hardly changed and the value was approximately 1.0 in RRM. GTP increased K_i values for dobutamine and

Fig. 2. Inhibition of specific ³H-DHA binding by β -agonists to turkey erythrocyte membranes (A) and the effect of guanine nucleotide (0.1 mM, GTP) (B). Displacement curves were obtained using various concentrations of β -agonists and 0.78 nM ³H-DHA. Each point represents the mean of 4–6 experiments, and each was performed in duplicate. A: ○, isoproterenol; ■, epinephrine; ▲, norepinephrine; ●, denopamine; △, dobutamine; □, dopamine; ◇, prenalterol; B: ○●, isoproterenol; △▲, dobutamine; ■, denopamine; ◇◆, prenalterol. Open and closed symbols represent the GTP absent and GTP present groups, respectively.

Table 2. Comparison of K_i values and Hill coefficients of β -adrenergic drugs determined by the inhibition of ³H-DHA binding to turkey erythrocyte membranes in the presence and absence of guanine nucleotide (GTP, 0.1 mM)

Drugs	N	GTP absent		GTP present	
		K _i (nM)	Hill	K _i (nM)	Hill
Agonists					
Isoproterenol	4	26.5 ± 4.8	0.767 ±0.022	170** ± 26	0.893*** ±0.019
Epinephrine	4	860 ± 160	0.760 ±0.038	3450** ± 510	0.874* ±0.014
Norepinephrine	4	360 ± 51	0.705 ±0.034	1650*** ± 150	0.939*** ±0.037
Denopamine	6	1540 ± 200	0.978 ±0.100	2610* ± 420	1.078 ±0.069
Dobutamine	4	1380 ± 220	0.791 ±0.029	2190** ± 77	0.958*** ±0.031
Dopamine	4	49500 ±2620	0.959 ±0.013	115520*** ±9370	1.069 ±0.043
Prenalterol	4	560 ± 23	0.770 ±0.062	1320** ± 150	1.083* ±0.082
Procaterol	4	5161 ± 674	0.888 ±0.079	5831 ± 532	0.967 ±0.099
Antagonist					
Practolol	4	4170 ± 340	0.946 ±0.039	4020 ± 540	0.999 ±0.049

* $P<0.05$, ** $P<0.02$, *** $P<0.01$, compared with the GTP absent group. Each datum represents the mean and S.E. of 4 or 6 experiments, each performed in duplicate determinations.

dopamine respectively by about 60 and 130% in TEM and by 100 and 150% in RRM.

Figure 4 shows schematic representation

of the effect of GTP on the K_i values in TEM and RRM. K_i values for full agonists like Iso, Epi and Nor increased in both membrane

Fig. 3. Inhibition of specific ^3H -DHA binding by β -agonists to the rat reticulocyte membranes (A) and the effect of guanine nucleotide (0.1 mM, GTP) (B). Displacement curves were obtained using various concentrations of β -agonists and 0.2 nM ^3H -DHA. Each point represents the mean of 4–5 experiments, and each was performed in triplicate. The lines through the data represent the best fit curves from the computer modeling procedures described in "Materials and Methods". A: $\circ$, isoproterenol; $\blacksquare$, epinephrine; $\blacktriangle$, norepinephrine; $\bullet$, denopamine; $\triangle$, dobutamine; $\square$, dopamine; $\diamond$, prenalterol; B: $\circ$, isoproterenol; $\triangle$, dobutamine; $\blacksquare$, denopamine; $\diamond$, prenalterol. Open and closed symbols represent the GTP absent and GTP present groups, respectively.

Table 3. Comparison of K_i values and Hill coefficients of β -adrenergic drugs determined by the inhibition of ^3H -DHA binding to rat reticulocyte membranes in the presence and absence of guanine nucleotide (GTP, 0.1 mM)

Drugs	N	GTP absent		GTP present	
		K _i (nM)	Hill	K _i (nM)	Hill
Agonists					
Isoproterenol	4	7.3 ± 0.5	0.688 ±0.019	41*** ± 2	0.824*** ±0.016
Epinephrine	4	58 ± 7	0.727 ±0.057	210** ± 30	0.810 ±0.048
Norepinephrine	4	1090 ± 150	0.735 ±0.028	3390*** ± 300	0.866* ±0.045
Denopamine	5	2300 ± 310	0.966 ±0.021	2120 ± 130	0.950 ±0.022
Dobutamine	4	750 ± 52	0.939 ±0.005	1420*** ± 33	1.027 ±0.039
Dopamine	4	26780 ±1030	0.793 ±0.027	64980*** ±4760	0.946*** ±0.009
Prenalterol	4	360 ± 25	1.013 ±0.043	370 ± 25	1.071 ±0.057
Procaterol	4	17.3 ± 2.9	0.990 ±0.058	35.5* ± 5.0	1.057 ±0.025
Antagonist					
Practolol	4	21920 ±1450	0.905 ±0.012	23590 ±2400	0.954 ±0.041

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, compared with the GTP absent group. Each datum represents the mean and S.E. of 4 or 5 experiments, each performed in triplicate determinations.

preparations and their arrows directed to the upper-right. The arrows of denopamine

directed only rightward, and the length of the X component was shorter than full agonists.

Fig. 4. Schematic representation of the effect of guanine nucleotide (0.1 mM, GTP) on the K_i values for the inhibition of specific ^3H -DHA binding to turkey erythrocyte membranes and rat reticulocyte membranes by β -adrenergic drugs. The X-axis represents the K_i values of β -adrenergic drugs for TEM and Y-axis, those for RRM. Closed and open symbols in the figure show the affinity of the drugs with and without GTP (0.1 mM), respectively. Therefore, the length of X and Y components of the arrows respectively show the degrees of the increase in K_i values in TEM (β_1) and RRM (β_2).

On the other hand, the arrow of the β_2 -selective agonist procaterol directed upward. In the case of practolol, a β_1 -antagonist, K_i values in both TEM and RRM were hardly influenced by GTP.

Discussion

The turkey erythrocyte membranes (TEM) and the rat reticulocyte membranes (RRM) possess β -agonist sensitive adenylate cyclase systems (10–12). TEM is known to be a homogeneous β_1 -adrenoreceptor model (10). However, RRM is only suggested to have a homogeneous population of β_2 -receptors, though the rat erythrocyte membranes possess homogenous β_2 -receptors (11–13). In our present experiment using RRM, Hofstee plots for both β_1 - and β_2 -selective drugs were almost linear (Fig. 1), and the computer analysis has demonstrated that RRM possesses a nearly homogeneous population of β_2 -receptor subtypes. When K_i values for the drugs tested in the present experiment in

TEM (β_1) and RRM (β_2) were compared with those in the rat heart (β_1) and lung (β_2) membranes (4), respectively, a good correlation was observed in the same receptor subtypes (TEM and rat heart: $r=0.998$, RRM and rat lung: $r=0.991$).

When the agonist (H) interacts with the receptor (R), both the low affinity (H–R) and high affinity complexes (H–R–N) are formed. Then, the N protein of the H–R–N complex is subjected to a conformational change and dissociates with GTP from the complex in the presence of GTP. Thus, the ternary high affinity complex (H–R–N) is reverted to the low affinity form (H–R). Therefore, the interaction of two affinity states is converted into that of a single population of receptor sites and the Hill coefficient approaches 1.0 by the addition of GTP (9, 16). In the present experiment, K_i values for Iso, Epi and Nor increased by about 300–500% in TEM and 200–460% in RRM, and Hill coefficients for these agonists also increased by the addition of GTP (Figs. 2 and 3, Tables 2 and 3). These results indicated that the agonist specific high affinity complex with receptors transformed to the low affinity complex as described above. It is well characterized that the higher the ability to form the high affinity complex with receptors, the stronger the intrinsic activity of agonists (8, 9).

We compared the difference between K_i values with and without GTP to estimate the ability to form the high affinity complex. The present experiment has demonstrated that denopamine exhibits agonist binding to the β_1 -receptor in TEM and has lower ability to form the high affinity complex than full agonists. This suggests that denopamine has lower intrinsic activity than full agonists at the β_1 -receptor. On the contrary, both the K_i value and Hill coefficient of denopamine for the inhibition curve in RRM were hardly changed by the addition of GTP, and its affinity (K_i) to β_2 -receptor was comparably low among the drugs tested. This infers that denopamine possesses extremely low or almost no agonist property at β_2 -receptors. In reference to this, Inamasu et al. (23) demonstrated that denopamine elevated the cellular cyclic AMP levels of the adipocytes

(β_1), but did not affect those of the diaphragm (β_2) and reticulocytes (β_2) in rats. Furthermore, Nagao et al. (2) reported that denopamine produced a very weak vasodilating effect, which was partly reduced by propranolol, showing a very weak agonistic action at β_2 -receptors. Our present experiment essentially corresponded with the above pharmacological experiments.

As for selectivity for β_1 - and β_2 -receptor subtypes, direct comparison of the K_i values for the two membrane preparations was not considered to be meaningful, because the two membrane sources are from two different animal species. Therefore, we tentatively drew a line in Fig. 4 dividing β_1 - and β_2 -selective drugs through the point for the non-selective β -agonist Iso without GTP in parallel with the diagonal line of identity.

Since dobutamine exhibited affinity for both β_1 - and β_2 -receptors and K_i values for both membranes were influenced by GTP, the drug was suggested to have agonist properties to both receptors. In dopamine, the K_i value and Hill coefficient were significantly influenced by the addition of GTP like dobutamine, but β -adrenergic action of this drug may be caused only at high concentration, since the affinities for TEM and RRM were extremely low. Prenalterol exhibited comparably high affinities and a similar binding characteristic to denopamine in both membrane preparations, although the β_1 -selectivity was lower than denopamine as shown in Fig. 4. In the previous paper (4), prenalteol hardly exhibited the agonist affinity to the rat heart membranes (β_1). Denopamine, on the other hand, showed a significant rightward shift of the competition curve by the addition of GTP in the same membrane preparation (4). This may be related to the tissue selectivity of prenalteol (24).

On the basis of the present findings, denopamine exhibited agonist affinity for TEM containing homogeneous β_1 -receptors, though the affinity of the drug for RRM (β_2) was comparably low and was hardly affected by a sufficient amount of GTP. This suggests that the drug possesses an agonist property at the β_1 -receptor and extremely low or almost no agonist property at the β_2 -receptor

and that the intrinsic activity at the β_1 -receptor is lower than full agonists. In this sense, denopamine is considered to have a characteristic of highly selective β_1 -adrenoceptor partial agonist in the binding experiments.

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