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

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Accepted September 2, 1985

Abstract—Affinities of denopamine, a new cardiotonic agent, and several \( \beta \)-adrenergic drugs for turkey erythrocyte membranes (TEM) and rat reticulocyte membranes (RRM) which contain homogeneous \( \beta_1 \)- and \( \beta_2 \)-receptors, respectively, were studied by receptor binding. The order of potencies of denopamine and several \( \beta \)-adrenergic agonists in displacing \( ^3 \mathrm{H} \)-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 \( \beta_1 \)-receptor but not at the \( \beta_2 \)-receptor and that the intrinsic activity at the \( \beta_1 \)-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 \( \beta_1 \)-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 rightward shift by GTP correspond to the intrinsic activities of \( \beta \)-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 \( \beta \)-receptors in the previous experiment may be obscured by the coexistence of \( \beta_1 \)- and \( \beta_2 \)-receptor subtypes in both membrane preparations. The present experiment was carried out to further investigate the agonist property of this drug to the \( \beta_1 \)- and \( \beta_2 \)-receptors using turkey erythrocyte membranes and rat reticulocyte membranes which were reported to contain homogeneous \( \beta_1 \)- and \( \beta_2 \)-receptors, respectively (10–13). In addition, we compared the degrees of the rightward shift by GTP among several \( \beta \)-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 NaHCO₃) 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 anticoagulant. After blood was centrifuged at 2000×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 MgCl₂, the lysate was centrifuged at 28000×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 μg/ml, respectively. In TEM, 10 mM MgCl₂ 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 (±)-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 IC₅₀ of radioligand competition curve was determined by the X-intercept of linearized plots of log [B/ Bₘₐₓ B] vs. log C, where B is the amount of ³H-DHA bound in the presence of competing drugs, Bₘₐₓ 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 (Ki) for the competing drugs were calculated from the IC50 by the following equation (17):

\[ K_i = IC50/(1 + L/K_d), \]

where L is the concentration of the radioligand in the incubation mixture, and Kd 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), \( \alpha \)-ascorbic acid, pargyline hydrochloride, phenylhydrazine hydrochloride, EDTA 2-Na and bovine serum albumin (Nakarai Chemicals).

| Table 1. | Equilibrium binding parameters for 3H-DHA to the rat erythrocyte and reticulocyte membranes and the turkey erythrocyte membranes |
|---|---|---|
| | Erythrocytes | Reticulocytes | Turkey |
| \( K_d \) (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±0.068 | 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 correlation coefficient for Scatchard or Hill plots. *P<0.01, compared with rat erythrocyte membranes.
selective antagonist practolol in RRM. Computer analysis has shown that the population of \( \beta_2 \)-receptors in RRM was calculated to be 99.5±0.5% (mean±S.E., \( N=4 \), procaterol) and 96.1±2.2% (practolol), indicating nearly homogeneous \( \beta_2 \)-receptors.

2. Effect of \( \beta \)-adrenergic drugs on \( ^{3} \)H-DHA binding to the turkey erythrocyte membranes (\( \beta_1 \)): Figure 2(A) and Table 2 show the displacement curves, inhibition constants (\( K_i \) values) and Hill coefficients of \( \beta \)-adrenergic drugs for \( ^{3} \)H-DHA binding to TEM. For typical catecholamines, the potency of the inhibition of \( ^{3} \)H-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 \( \beta_1 \)-adrenoceptor subtype (22). The potency of the binding inhibition of other drugs in TEM was in the order of 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 \( \beta_1 \)- and \( \beta_2 \)-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 \( \beta_1 \)-partial agonist, and practolol, a \( \beta_1 \)-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.

3. Effects of \( \beta \)-adrenergic drugs on \( ^{3} \)H-DHA binding to the rat reticulocyte membranes (\( \beta_2 \)): Displacement curves and \( K_i \) values for \( \beta \)-adrenergic drugs in RRM are shown in Fig. 3(A) and Table 3. The potency of the inhibition of the \( ^{3} \)H-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 \( \beta_2 \)-subtype (22). In other drugs, the potency of the \( ^{3} \)H-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 \( \beta_2 \)-receptor was comparably low. Practolol, a \( \beta_1 \)-antagonist, showed a very low affinity among the drugs tested.

4. Effects of GTP on the inhibition of \( ^{3} \)H-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 \( \beta_1 \)- and \( \beta_2 \)-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 \( \beta_1 \)-partial agonist, and practolol, a \( \beta_1 \)-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
dopamine respectively by about 60 and 130% in TEM and by 100 and 150% in RRM.

Figure 2. Inhibition of specific $^3$H-DHA binding by $\beta$-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 $\beta$-agonists and 0.78 nM $^3$H-DHA. Each point represents the mean of 4–6 experiments, and each was performed in duplicate. A: $\bigcirc$, isoproterenol; $\blacksquare$, epinephrine; $\blacktriangle$, norepinephrine; $\bullet$, denopamine; $\Delta$, dobutamine; $\bigcirc$, dopamine; $\bigtriangleup$, prenalterol; B: $\bigcirc\bigcirc$, isoproterenol; $\bigtriangleup\bigtriangleup$, dobutamine; $\blacksquare\blacksquare$, denopamine; $\bigstar\bigstar$, 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 $\beta$-adrenergic drugs determined by the inhibition of $^3$H-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
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.

**Table 3.** Comparison of $K_i$ values and Hill coefficients of $\beta$-adrenergic drugs determined by the inhibition of $^3$H-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.

![Image of Figure 3](image-url)

**Fig. 3.** Inhibition of specific $^3$H-DHA binding by $\beta$-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 $\beta$-agonists and 0.2 nM $^3$H-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: ○, 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.
Fig. 4. Schematic representation of the effect of guanine nucleotide (0.1 mM, GTP) on the Ki 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 Ki 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 Ki values in TEM (¥) and RRM (ß).

On the other hand, the arrow of the β₂-selective agonist procaterol directed upward. In the case of practolol, a β₁-antagonist, Ki 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-adrenergic sensitive adenylate cyclase systems (10–12). TEM is known to be a homogeneous β₁-adrenoceptor model (10). However, RRM is only suggested to have a homogeneous population of β₂-receptors, though the rat erythrocyte membranes possess homogenous β₂-receptors (11–13). In our present experiment using RRM, Hofstee plots for both β₁- and β₂-selective drugs were almost linear (Fig. 1), and the computer analysis has demonstrated that RRM possesses a nearly homogeneous population of β₂-receptor subtypes. When Ki values for the drugs tested in the present experiment in TEM (β₁) and RRM (β₂) were compared with those in the rat heart (β₁) and lung (β₂) 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, Ki 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 Ki 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 β₁-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 β₁-receptor. On the contrary, both the Ki value and Hill coefficient of denopamine for the inhibition curve in RRM were hardly changed by the addition of GTP, and its affinity (Ki) to β₂-receptor was comparably low among the drugs tested. This infers that denopamine possesses extremely low or almost no agonist property at β₂-receptors. In reference to this, Inamasu et al. (23) demonstrated that denopamine elevated the cellular cyclic AMP levels of the adipocytes.
(β₁), but did not affect those of the dia-
phragm (β₂) and reticulocytes (β₂) 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 β₂-receptors. Our
present experiment essentially corresponded
with the above pharmacological experiments.

As for selectivity for β₁- and β₂-receptor
subtypes, direct comparison of the Kᵢ 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 β₁- and β₂-
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 β₁- and β₂-receptors and Kᵢ values for
both membranes were influenced by GTP,
the drug was suggested to have agonist
properties to both receptors. In dopamine, the
Kᵢ 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 concen-
tration, 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 β₁-
selectivity was lower than denopamine as
shown in Fig. 4. In the previous paper (4),
prenalterol hardly exhibited the agonist
affinity to the rat heart membranes (β₁).
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 prenalterol
(24).

On the basis of the present findings,
denopamine exhibited agonist affinity for
TEM containing homogeneous β₁-receptors,
though the affinity of the drug for RRM (β₂)
was comparably low and was hardly affected
by a sufficient amount of GTP. This suggests
that the drug possesses an agonist property
at the β₁-receptor and extremely low or
almost no agonist property at the β₂-receptor
and that the intrinsic activity at the β₁-receptor
is lower than full agonists. In this sense,
denopamine is considered to have a charac-
teristic of highly selective β₁-adrenoceptor
partial agonist in the binding experiments.

Acknowledgement: We thank Mr. M. Inamasu for
valuable discussions, and we also thank Miss Y.
Yamaguchi for technical assistance.

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