Effects of Chronic Administration of Carteolol on \( \beta \)-Adrenoceptors in Spontaneously Hypertensive Rat Heart

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ABSTRACT—We studied the effects of chronic administration of \( \beta \)-adrenoceptor antagonists with and without intrinsic sympathomimetic activity (ISA): carteolol (with ISA) and propranolol (without ISA), respectively, on the heart of spontaneously hypertensive rat (SHR) and Wistar Kyoto rat (WKY). Six-week-old SHRs and WKYs were orally given carteolol or propranolol for ten weeks. The heart rate was reduced in propranolol-treated SHR, but not in carteolol-treated ones. In WKY, carteolol treatment increased the heart rate. The number and affinities of \( \beta \)-adrenoceptors were analyzed using \(^3\text{H}\)dihydroalprenolol as a ligand. Propranolol at 30 mg/kg increased the number of cardiac \( \beta \)-adrenoceptors in both SHR and WKY. In contrast, 10 mg/kg carteolol significantly decreased the number of cardiac \( \beta \)-adrenoceptors in SHR, but not in WKY. These data indicate that carteolol, a \( \beta \)-adrenoceptor antagonist with ISA, does not cause up-regulation of the number of cardiac \( \beta \)-adrenoceptors in the rat and suggest that this fact is related to a possible lack of “rebound phenomena” after sudden discontinuation of chronic carteolol-therapy in humans.

Propranolol is a \( \beta \)-adrenoceptor antagonist which is widely used in the treatment of diseases such as hypertension and angina pectoris. However, a number of untoward cardiac events, in particular “rebound phenomena”, occurred after abrupt withdrawal of propranolol-therapy (1–3). By radioligand binding studies, several investigators have demonstrated that chronic in vivo treatment with propranolol leads to a significant increase in the number of \( \beta \)-adrenoceptors in rat hearts (4, 5). It has been suggested that this increase in the number of \( \beta \)-adrenoceptors as a result of chronic propranolol treatment is associated with the elevated sensitivity to catecholamines (6, 7). Such findings would provide a possible explanation for the rebound phenomena after withdrawal of chronic propranolol-therapy in humans.

Several investigators found that in human lymphocytes, chronic treatment with propranolol led to an increase in the number of \( \beta \)-adrenoceptors, while chronic treatment with \( \beta \)-adrenoceptor antagonists with intrinsic sym-
pathomimetic activity (ISA) did not lead to an increase (but rather led to a decrease) in the number of $\beta$-adrenoceptors (8–10). Based on these results, they suggested that “rebound phenomena” could be prevented by the use of $\beta$-adrenoceptor antagonists with ISA. Thus, the ISA may play an important role in $\beta$-adrenoceptor antagonist-induced regulation of the number of $\beta$-adrenoceptors.

Carteolol is a nonselective $\beta$-adrenoceptor antagonist with ISA (11) used clinically for mild to moderate hypertension, and this antagonist could cause vasodilation by facilitation of the release of endothelium-derived relaxing factor (12) and by a presynaptic inhibitory action on adrenergic nerve ending (13, 14). To our knowledge, there is no report regarding the rebound phenomena after withdrawal of chronic carteolol-therapy. To date, several reports regarding regulation of $\beta$-adrenoceptors after chronic treatment of $\beta$-adrenoceptor antagonists with ISA have appeared (15–18). However, the relationship between the ISA of these drugs and changes in physiological parameters such as blood pressure and heart rate during chronic treatment has not been fully characterized.

Therefore, we studied the effects of chronic carteolol administration on the number of cardiac $\beta$-adrenoceptors in spontaneously hypertensive rat (SHR) and Wistar Kyoto rat (WKY). The systolic blood pressure and heart rate of the same animals were measured in an attempt to correlate their physiological responses with the ISA of this drug during chronic treatment.

MATERIALS AND METHODS

Chronic drug treatment

Male SHRs and WKYs supplied by Charles River Japan, Inc. were used. Since carteolol and propranolol are usually administered orally to humans, we also gave these drugs to the animals by the oral route. Six-week-old SHRs were orally given, twice a day, carteolol (0.3, 3, 10 mg/kg/day) or propranolol (30 mg/kg/day) dissolved in water for 10 weeks as the $\beta$-blocking effect of 0.3 mg/kg carteolol on isoproterenol-stimulated SHR heart rate was seen for about 12 hours (19). Age-matched WKYs were orally given carteolol (10 mg/kg/day) or propranolol (30 mg/kg/day). Control SHR and WKY rats were given corresponding volumes of water. Both systolic blood pressure and heart rate were measured at 16 hr after drug administration by the tail-cuff method. Sixteen hours after the last dose, the animals were anesthetized with ether, sacrificed by exsanguination, and then the hearts were removed for radioligand binding studies.

Tissue preparation

Hearts were carefully removed from the animals, rinsed with ice-cold saline, and then kept in a freezer (−80°C). The ventricles were minced with scissors and homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with a glass homogenizer. The homogenates were filtered through two layers of gauze, re-homogenized two times at setting 10 on a polytron (PT-10) with 20-second bursts, centrifuged at 1,000 × g for 10 min, and then the supernatant was carefully removed and centrifuged at 100,000 × g for 30 min. The resulting pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.4). Protein concentration was determined by the method of Lowry et al. (20).

Binding assay

The binding assays using $[^3]$H)dihydroalprenolol ($[^3]$HDHA) as the radioligand were performed by incubating aliquots of the heart homogenates at a temperature of 25°C for 20 min in 250 μl of the Tris-HCl buffer, containing $[^3]$HDHA, in the absence or presence of a high concentration of alprenolol (10 μM). The binding in the presence of 10 μM alprenolol, the non-specific binding, was subtracted from that obtained in the absence of alprenolol, the total binding, to obtain the specific binding. The assay was terminated by the addition of 3 ml of ice-cold buffer followed by rapid filtration through the Whatmann GF/C glass fiber filters under suction. After washing twice with
3 ml of the buffer, the filters were transferred to counting vials and 5 ml of scintillation fluid was added. Radioactivity was counted in a Packard Tri-Carb scintillation spectrometer (model 2000 CA). All assays were performed in triplicate.

**Drugs used**

[3H]DHA (specific activity, 92.2 Ci/mmol) was purchased from New England Nuclear, Boston, MA, U.S.A. 1-Alprenolol d-tartrate and dl-propranolol hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. dl-Carteolol hydrochloride was obtained from Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan. All other chemicals were of reagent grade or of the purest grade commercially available.

**Statistical analysis**

The experimental data given in the text are means ± S.E. of N experiments. The significance of difference was estimated by Dunnett's test, and values of P < 0.05 were considered statistically significant. Comparisons of the changes of systolic blood pressure and heart rate were performed between pre- (6-week old) and drug-administration (8-, 12- and 16-week old, respectively). In the radio-

**RESULTS**

**Effects of carteolol and propranolol on systolic blood pressure and heart rate**

In SHR, the oral administration of carteolol (0.3, 3 and 10 mg/kg) and propranolol (30 mg/kg) for 10 weeks did not significantly affect systolic blood pressure (Fig. 1). The ISA of carteolol and propranolol was determined by changes in the heart rate of SHR. Propranolol (30 mg/kg) produced a significant decrease in the heart rate of 12-week and 16-week old rats (drug administration for 6 and 10 weeks, respectively) (Fig. 2). Carteolol (0.3 and 3 mg/kg) had a tendency to reduce the heart rate, but the changes were not statistically significant.

In WKY, the administration of carteolol (10 mg/kg) and propranolol (30 mg/kg) for 10 weeks did not have any significant influence on systolic blood pressure; however, carteolol (10 mg/kg) produced a significant increase in the heart rate of 16-week old rats (Table 1).

![Fig. 1. Age-related changes in systolic blood pressure (SBP) in SHR. SBP was measured by the tail-cuff method. Drugs were administered as described in Materials and Methods: (●) carteolol (0.3 mg/kg/day) (n = 13), (■) carteolol (3 mg/kg/day) (n = 15), (●) carteolol (10 mg/kg/day) (n = 14), (▲) propranolol (30 mg/kg/day) (n = 11). Control animals (○) (n = 13) were given the corresponding volume of water. The values are given as means ± S.E. Statistical analyses were performed by Dunnett's test.](image-url)
Table 1. Effects of carteolol and propranolol on systolic blood pressure (SBP) and heart rate (HR) in 6-week and 16-week old WKY

<table>
<thead>
<tr>
<th></th>
<th>6-week old (no drug)</th>
<th>6-week old (10 mg/kg carteolol)</th>
<th>16-week old (no drug)</th>
<th>16-week old (30 mg/kg propranolol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP (mmHg)</td>
<td>HR (beats/min)</td>
<td>SBP (mmHg)</td>
<td>HR (beats/min)</td>
</tr>
<tr>
<td>Control</td>
<td>115 ± 3</td>
<td>405 ± 8</td>
<td>122 ± 2</td>
<td>305 ± 5</td>
</tr>
<tr>
<td>Carteolol (10 mg/kg)</td>
<td>112 ± 3</td>
<td>398 ± 6</td>
<td>125 ± 3</td>
<td>328 ± 4**</td>
</tr>
<tr>
<td>Propranolol (30 mg/kg)</td>
<td>104 ± 2</td>
<td>385 ± 4</td>
<td>112 ± 2</td>
<td>282 ± 6</td>
</tr>
</tbody>
</table>

SBP and HR were measured by the tail-cuff method. Drugs were administered as described in Materials and Methods. The values are given as means ± S.E. (N = 15 – 16). **P < 0.01, significantly different from the respective control value.

Characterization of [3H]DHA binding to β-adrenoceptors in SHR heart

Specific [3H]DHA binding increased linearly with increasing protein concentration up to 0.4 mg protein per assay (data not shown). Therefore, binding assays were conducted with less than 0.4 mg of protein per assay. At 25°C, specific [3H]DHA binding was rapid, reached equilibrium within 15 min and was displaced by the addition of 10 μM alprenolol (Fig. 3). The specific binding of increasing concentration of [3H]DHA (1.3–14.0 nM) was saturable (Fig. 4). Scatchard analysis indicated a single class of binding sites. A typical Scatchard plot is shown in the inset of Fig. 4.

Effects of chronic administration of carteolol and propranolol on β-adrenoceptors

In SHR, 10 mg/kg carteolol significantly decreased the number of β-adrenoceptors. In contrast, 30 mg/kg propranolol significantly increased the number of β-adrenoceptors (Table 2). In WKY, 10 mg/kg carteolol did not significantly affect the number of β-adre-
noceptors and 30 mg/kg propranolol significantly increased the number of $\beta$-adrenoceptors (Table 3). There was no significant difference in the $K_d$ values of the control and drug-treated groups in both SHR and WKY hearts. There was also no significant difference in wet weight of the ventricles among the groups (data not shown).
DISCUSSION

In the present study, we observed a decrease in heart rate in propranolol (30 mg/kg)-treated SHR, but not in carteolol (10 mg/kg)-treated SHR. Although the $\beta$-blocking potency of carteolol was 30 to 300 times larger than that of propranolol (11, 19), the decrease in heart rate was not observed in carteolol-treated SHR, suggesting that the ISA of this drug masked the $\beta$-blocking effect on the heart rate. A tendency for the heart rate to decrease following smaller doses (0.3 and 3 mg/kg) of carteolol may be due to the weaker ISA in such doses. In fact, the heart rate was increased in carteolol (10 mg/kg)-treated 16-week old WKY.

In the radioligand binding studies using $[^3H]$DHA, there was no difference in the number of cardiac $\beta$-adrenoceptors between the SHR control and WKY control (Tables 2 and 3). This finding is in agreement with several other reports (22–24). On the other hand, it has been reported that the number of cardiac $\beta$-adrenoceptors in SHR is smaller than that in WKY (25–27). The reason for this discrepancy remains unknown.

We observed an increase in the number of $\beta$-adrenoceptor binding site in the heart taken from 30 mg/kg propranolol-treated SHR and WKY, without any significant changes in the $K_d$ values. Such increases in the number of cardiac $\beta$-adrenoceptors are in agreement with other reports (4, 5). On the other hand, 0.3 mg/kg carteolol (which is nearly equipotent to 30 mg/kg propranolol) had no effect on $\beta$-adrenoceptors in SHR heart, and 10 mg/kg carteolol decreased the number of $\beta$-adrenoceptors in SHR heart without significant changes in the $K_d$ values. Our results are in agreement with those of other investigators who suggested that the "rebound phenomena"
after withdrawal would not occur following $\beta$-adrenoceptor antagonists with ISA (8–10). In WKY, we observed a significant increase in heart rate at 10 weeks following 10 mg/kg carteolol (16-week old), suggesting that the ISA was actually acting in carteolol-treated WKY. However, we could not observe any significant decrease in the number of $\beta$-adrenoceptor binding sites in such hearts (Table 3). These findings suggest that cardiac $\beta$-adrenoceptors in SHR may be more sensitive to ISA-induced down-regulation of receptor number than that in WKY. It is well-established that physiological conditions of hypertensive rats are quite different from those of normotensive rats (14, 24, 28–30). For example, the $\beta_1: \beta_2$-adrenoceptor ratio in the heart and lung of SHR is significantly different from that of WKY (24) and presynaptic $\beta$-adrenoceptors may function tonically to facilitate the release of norepinephrine in the renal arteries of young SHR more than that of age-matched WKY (14). It is considered that the regulation of the receptor number in SHR may be different from that in WKY. Further studies at the molecular level are required to understand this difference in regulation mechanism between cardiac $\beta$-adrenoceptors of SHR and WKY.

In conclusion, carteolol, a $\beta$-adrenoceptor antagonist with ISA, did not cause the up-regulation of cardiac $\beta$-adrenoceptors in SHR, but produced a decrease in the number of receptors. This decrease may be related to a possible lack of a "rebound phenomena" after sudden discontinuation of chronic carteolol-therapy in humans.

REFERENCES

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