MODULATION OF NORADRENALINE RELEASE VIA ACTIVATION OF PRESYNAPTIC β-ADRENOCEPTORS IN RABBITS WITH ADRIAMYCIN-INDUCED CARDIOMYOPATHY

YOSHIHIRO UNO, M.D., SHINYA MINATOGUCHI, M.D., YOKO IMAI, M.D.
MASATOSHI KOSHIJI, M.D., MASAO KAKAMI, M.D.
HITOMI YOKOYAMA, M.D., HIROYASU ITO, M.D.
AND SENRI HIRAKAWA, M.D.

We investigated the role of β-adrenoceptors at postganglionic sympathetic nerve endings in noradrenaline release in rabbits with cardiomyopathic congestive heart failure produced by adriamycin (1 mg/kg, I.V., twice a week for 8 weeks). Plasma noradrenaline levels were measured before, 30 min after, and 60 min after the start of continuous intravenous administration of adrenaline (0.06 μg/kg/min) in adriamycin-treated and vehicle-treated rabbits in anesthetized condition and pithed condition with electrically stimulated sympathetic outflow (3 Hz, 1 ms square wave pulse, 90 V). In both the anesthetized and pithed conditions, adrenaline increased plasma noradrenaline levels in vehicle-treated rabbits. However, in the adriamycin-treated rabbits, adrenaline had no effect on the plasma noradrenaline level. Pretreatment with propranolol (0.2 mg/kg, bolus I.V. + 0.1 mg/kg/hr, continuous infusion) almost completely abolished the rise in plasma noradrenaline associated with adrenaline infusion in vehicle-treated rabbits. These results suggest that in rabbits with adriamycin-induced cardiomyopathy, the noradrenaline release from the sympathetic nerve endings via the activation of presynaptic β-adrenoceptors is reduced. This might be due to down-regulation of presynaptic β-adrenoceptors caused by the elevated plasma noradrenaline due to cardiac failure. However, other possibilities such as reduced affinity or impaired signal transduction cannot be excluded.

(Jpn Circ J 1993; 57: 426—433)

It is well known that sympathetic nerve activity is enhanced1 and the plasma noradrenaline level is increased2,3 as the result of compensatory mechanisms in patients with congestive heart failure. Elevated plasma noradrenaline is believed to induce down regulation of β-adrenoceptors in the myocytes in congestive heart failure4,5. In rabbits with adriamycin-induced cardiomyopathic congestive heart failure, whether the number of cardiac β-adrenoceptors is reduced may depend on the doses and the duration of adriamycin treatment6,7. Both α- and β-adrenoceptors are present at the sympathetic nerve endings. Activation of the β-adrenoceptors at the sympathetic nerve ending can enhance the release of noradrenaline8,9 and activation of α-adrenoceptors can inhibit the release of noradrenaline. Adriamycin in a regimen of 1 mg/kg,

Key words: Presynaptic β-adrenoceptors Adriamycin-induced cardiomyopathy Adrenaline Noradrenaline Pithed rabbit

(Received July 2, 1992; accepted October 26, 1992)
The 2nd Department of Internal Medicine Gifu University School of Medicine 40 Tsukasa Machi, Gifu 500, Japan Mailing address: Yoshihiro Uno M.D., The 2nd Department of Internal Medicine Gifu University School of Medicine 40 Tsukasa Machi Gifu 500, Japan

426 Japanese Circulation Journal Vol.57, May 1993
Presynaptic β-adrenoceptors in Cardiomyopathy

Completion of Set Up

<table>
<thead>
<tr>
<th>Completion of Set Up</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>⊗</td>
<td>⊗</td>
<td>⊗</td>
</tr>
<tr>
<td>20</td>
<td>⊗</td>
<td>⊗</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>⊗</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 (min)</td>
<td>⊗</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adrenaline (0.06 μg/kg/min) or Saline continuous infusion

Anesthesia with Pentobarbital or Pithing with Electrically Stimulated Sympathetic Outflow (80V, 3 Hz, 1 m sec)

Fig.1. Time schedule of the experiments ⊗ represents blood sampling point.

twice weekly for 8 weeks has been proven to produce low cardiac output cardiomyopathy without impairing the function of presynaptic α2-adrenoceptors, activation of which can inhibit the release of noradrenaline from the sympathetic nerve endings. This suggests that the dose of adriamycin used does not damage presynaptic α2-adrenoceptors. However, the role of β-adrenoceptors at the sympathetic nerve endings in the release of noradrenaline in adriamycin-treated rabbits is unknown.

The aim of the present study was to investigate the role of presynaptic β-adrenoceptors at the sympathetic nerve endings in releasing noradrenaline in rabbits with adriamycin-induced cardiomyopathy.

MATERIALS AND METHODS

1) Rabbits with adriamycin-induced cardiomyopathy

We treated 10- to 12-week-old male Japanese White rabbits (mean body weight ±SE: 2.5±0.1kg) with adriamycin (1 mg/kg, I.V., twice a week) via a marginal ear vein for 8 weeks, according to the method described by McGrath et al.11,12

2) Anesthetized rabbit

Rabbits were anesthetized with sodium pentobarbital (70 mg/kg, I.V.) via a marginal ear vein. The trachea was cannulated and artificial respiration was maintained with a respirator (Natsume Seisakujo Co., Ltd.; type 60). The left carotid artery was cannulated with polyethylene tubing which was connected to a blood pressure transducer (American Gould Co., Ltd.; P 23 10) for the measurement of blood pressure and heart rate with a polyrecorder (Nihon Kohden Co., Ltd.; STC-502). The left jugular vein was also cannulated with polyethylene tubing, which was inserted as far as the right atrium, through which either drug or saline was injected. A 2.5 F thermistor catheter (American Edwards Laboratories Co., Ltd.; 94-030-2.5F) was inserted through the left femoral artery to measure cardiac output by the thermodilution method. The tip of the catheter was positioned in the abdominal aorta just below the renal artery (~13 cm). Cardiac output was measured after the injection of 1 ml of cold saline (4°C) into the right jugular vein by a cardiac output computer (AHS Japan Co., Ltd.; COC-9520). The average of three measurements was taken as the reading. After cardiac output was measured, the thermistor catheter was removed and replaced with polyethylene tubing through which blood samples were taken for measurements of plasma catecholamine concentrations. Maintenance anesthesia (15 mg/kg/h, I.V.) was then initiated through an ear vein. At autopsy after the experiments, all cannulations were found to have been correctly done.

3) Pithed rabbits

Rabbits were anesthetized and maintained on artificial respiration as described above. The right and left carotid arteries and right and left jugular veins were cannulated with polyethylene tubing. A small hole was made in the parietal bone and a stainless steel rod was inserted 17 cm down the spine as measured from the hole. The rod was 4 mm in diameter and was electrically insulated by covering with plastic tape except for a 2 cm section at the tip of the rod. The indifferent electrode was inserted into the muscle at the back of the neck. Tubocurarine chloride (1 mg/kg, I.V.) was administered to prevent skeletal muscle contraction. Continuous electrical stimulation was initiated at a frequency of 3 Hz with 1 ms square wave pulses and at a voltage of 90 V in order to maintain blood pressure.

Japanese Circulation Journal  Vol.57, May 1993
TABLE I HEMODYNAMIC PARAMETERS, BODY WEIGHT AND HEART WEIGHT

<table>
<thead>
<tr>
<th></th>
<th>Vehicle-treated (n=27)</th>
<th>Adriamycin-treated (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (kg)</strong></td>
<td>2.49±0.03</td>
<td>2.58±0.04</td>
</tr>
<tr>
<td><strong>Heart Weight (g)</strong></td>
<td>3.5±0.20</td>
<td>6.8±0.18**</td>
</tr>
<tr>
<td><strong>Heart Weight/Body Weight (g/kg)</strong></td>
<td>2.22±0.07</td>
<td>2.62±0.06**</td>
</tr>
<tr>
<td><strong>Cardiac Output (l/min)</strong></td>
<td>0.427±0.015</td>
<td>0.362±0.012*</td>
</tr>
<tr>
<td><strong>Mean Arterial Pressure (mmHg)</strong></td>
<td>65.0±3.3</td>
<td>65.3±3.1</td>
</tr>
<tr>
<td><strong>Heart Rate (beats/min)</strong></td>
<td>248±10</td>
<td>197±9**</td>
</tr>
</tbody>
</table>

Results were expressed as mean±SE. Significant difference as compared with the Vehicle-treated group

\* = p<0.05, \*\* = p<0.01

4) Schedule of experiment

As shown in Fig. 1, we defined the time point when all instrumentations had been finished as 0 min (t=0). Adrenaline (0.06 μg/kg/min) or saline infusion was initiated after taking a 20 min rest from the time point t=0. The first 4 ml blood sample (S1) was taken from the femoral artery immediately before the start of administration of adrenaline or saline. Further blood samples were taken at 50 min (4 ml, S2) and at 80 min (4 ml, S3). After each blood sample was taken, the rabbits were given dextran (10%) via the femoral artery in an amount equal to half the volume of the blood withdrawn. Plasma noradrenaline and adrenaline concentrations were determined by high performance liquid chromatography coupled with trihydroxyindole fluorimetric detection. Because individual variations of plasma catecholamine levels were relatively large, the effect of drugs on the plasma noradrenaline levels and hemodynamic parameters were assessed by the mean value of percent changes at S2 and S3. [S2% change=(S2 value/S1 value)×100, S3% change=(S3 value/S1 value)×100]

5) Groups of the rabbits studied

A) Anesthetized rabbits
   a) Vehicle-treated group
      (1) saline was infused (n=7).
      (2) adrenaline (0.06 μg/kg/min) was infused (n=7).
   b) Adriamycin-treated group
      (1) saline was infused (n=7).
      (2) adrenaline (0.06 μg/kg/min) was infused (n=7).
   c) Vehicle-treated group pretreated with propranolol
      (0.2 mg/kg bolus injection + 0.1 mg/kg/h infusion).
      (1) saline was infused (n=7).
      (2) adrenaline (0.06 μg/kg/min) was infused (n=7).

B) Pithed rabbits with electrically stimulated sympathetic outflow
   a) Vehicle-treated group
      (1) saline was infused (n=6).
      (2) adrenaline (0.06 μg/kg/min) was infused (n=7).
   b) Adriamycin-treated group
      (1) saline was infused (n=7).
      (2) adrenaline (0.06 μg/kg/min) was infused (n=6).

6) Statistical analysis

Data are all expressed as mean±SE. Statistical significance was determined by Student’s t-test and two way analysis of variance.

RESULTS

1) Effects of adriamycin treatment on hemodynamic parameters and heart weight

The hemodynamic parameters showed in Table I were obtained at time point t=0, when neither saline nor adrenaline had been administrated in all the subgroups, except for the propranolol pretreated group under conditions of anesthesia. There was no significant difference in body weight between the vehicle-treated and the adriamycin-treated groups (Table I). However, both heart weight and ratio of heart weight to body weight were significantly greater in the adriamycin-treated group than in the vehicle-

*Japanese Circulation Journal Vol.57, May 1993*
Presynaptic \( \beta \)-adrenoceptors in Cardiomyopathy

TABLE II PLASMA CATECHOLAMINE LEVELS IN ANESTHETIZED AND PITTED RABBIT

<table>
<thead>
<tr>
<th></th>
<th>Vehicle-treated</th>
<th>Adriamycin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( (n=14) )</td>
<td>( (n=14) )</td>
</tr>
<tr>
<td>Anesthetized Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline (ng/ml)</td>
<td>0.239±0.028</td>
<td>0.333±0.037*</td>
</tr>
<tr>
<td>Adrenaline (ng/ml)</td>
<td>0.115±0.021</td>
<td>0.132±0.032</td>
</tr>
<tr>
<td>Pithed Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline (ng/ml)</td>
<td>0.278±0.052</td>
<td>0.505±0.091*</td>
</tr>
<tr>
<td>Adrenaline (ng/ml)</td>
<td>0.194±0.087</td>
<td>0.408±0.124</td>
</tr>
</tbody>
</table>

Results were expressed as mean±SE. Significant difference as compared with the Vehicle-treated group. * = \( p < 0.05 \)

TABLE III CHANGES IN HEART RATE AND MEAN BLOOD PRESSURE DUE TO THE INFUSION OF SALINE AND ADRENALINE

<table>
<thead>
<tr>
<th></th>
<th>Vehicle-treated</th>
<th>Adriamycin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( (n=7) )</td>
<td>( (n=7) )</td>
</tr>
<tr>
<td>Anesthetized Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate (%)</td>
<td>94.5±2.1</td>
<td>93.9±1.4</td>
</tr>
<tr>
<td>Mean Blood Pressure (%)</td>
<td>102.5±1.2</td>
<td>110.3±5.6</td>
</tr>
<tr>
<td>( (n=7) )</td>
<td>( (n=7) )</td>
<td></td>
</tr>
<tr>
<td>Pithed Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate (%)</td>
<td>88.9±3.8</td>
<td>82.7±7.0</td>
</tr>
<tr>
<td>Mean Blood Pressure (%)</td>
<td>107.7±7.2</td>
<td>129.0±5.3*</td>
</tr>
<tr>
<td>( (n=6) )</td>
<td>( (n=6) )</td>
<td></td>
</tr>
</tbody>
</table>

Results were expressed as mean±SE. Significant difference as compared with saline group. * = \( p < 0.05 \). Statistical significance was assessed by two way analysis of variance.

trated group. In rabbits treated with adriamycin, cardiac output and heart rate were significantly lower than in the vehicle-treated group. There was no significant difference in mean blood pressure between the two groups.

2) Plasma noradrenaline and adrenaline levels

In anesthetized adriamycin-treated rabbits, plasma noradrenaline levels at time point S1 were significantly higher than in the vehicle-treated group (Table II). In pithed rabbits with electrically stimulated sympathetic outflow, there was the same increase in plasma noradrenaline levels in adriamycin-treated rabbits as there was in vehicle-treated rabbits (Table II). In contrast, there were no significant differences in plasma adrenaline levels between vehicle-treated or adriamycin-treated rabbits in the anesthetized or pithed groups (Table II).

3) Effects of adrenaline infusion on mean blood pressure and heart rate

Continuous infusion of adrenaline did not affect heart rate in adriamycin-treated or vehicle-treated rabbits in the anesthetized or the pithed groups (Table III). In vehicle-treated rabbits, adrenaline increased mean blood pressure in the pithed condition, but not in the anesthetized condition. In adriamycin-treated rabbits, adrenaline did not affect mean blood pressure in either the anesthetized or the pithed condition (Table III).

4) Effects of adrenaline infusion on the plasma noradrenaline level

Fig. 2 shows the percent changes in the plasma noradrenaline levels due to continuous infusion of adrenaline in the vehicle-
treated group and the adriamycin-treated group under both anesthetized (upper panel) and pithed (lower panel) conditions. The columns represent the percent changes of the mean value at 30 min (S2/S1) and at 60 min (S3/S1) after the start of adrenaline or saline infusion. In anesthetized rabbits (Fig. 2-a), adrenaline significantly increased plasma noradrenaline levels (91.6 ± 13.4 vs 136.0 ± 19.5%) as compared with saline infused controls in the vehicle-treated group. On the other hand, in the adriamycin-treated group, adrenaline infusion did not significantly increase the plasma noradrenaline levels. In the pithed rabbits with electrically stimulated sympathetic outflow, adrenaline infusion significantly increased plasma noradrenaline levels as compared with saline infused controls in the vehicle-treated group (94.0 ± 5.0 vs 142.7 ± 28.9%; Fig. 2-b), but not in the adriamycin-treated group.

5) Effects of adrenaline infusion on the plasma noradrenaline levels after pretreatment with propranolol

In anesthetized vehicle-treated rabbits, pretreatment with propranolol completely abolished the adrenaline-related increase in plasma noradrenaline levels (Fig. 3).

DISCUSSION

Chronic treatment with adriamycin (1 mg/kg, I.V., twice a week for 8 weeks) has

Japanese Circulation Journal Vol.57, May 1993
been found to produce low-output cardiac failure in rabbits.\textsuperscript{11,12} In our study, the same chronic treatment with adriamycin, in the same dosage, produced signs of cardiac failure such as reduced cardiac output, increased heart weight, increased heart weight to body weight ratio, and increased plasma noradrenaline levels. Adrenaline infused at a dose of 0.06 µg/kg/min, has been found to selectively activate \( \beta \)-adrenoceptors at postganglionic sympathetic nerve endings in anesthetized rabbits, thus enhancing noradrenaline release into the plasma from the sympathetic nerve endings.\textsuperscript{9}

In our study, adrenaline infusion (0.06 µg/kg/min) significantly increased plasma noradrenaline levels in anesthetized vehicle-treated rabbits. This effect was almost completely abolished by pretreatment with propranolol in anesthetized vehicle-treated rabbits. These findings suggest that the adrenaline-induced increase in plasma noradrenaline level in anesthetized and vehicle-treated rabbits resulted from activation of \( \beta \)-adrenoceptors in the central nervous system and/or at the sympathetic nerve endings, since this dose of adrenaline infusion reportedly does not affect noradrenaline clearance in the plasma.\textsuperscript{9}

In the pithed rabbits with electrically stimulated sympathetic outflow, where central nervous system effects are eliminated, continuous infusion of adrenaline significantly increased the plasma noradrenaline level in vehicle-treated rabbits (Fig. 2). However, in adriamycin-treated rabbits, adrenaline infusion had no effect on the plasma noradrenaline level in anesthetized or pithed rabbits (Fig. 2). These results suggest that noradrenaline release, induced by the activation of \( \beta \)-adrenoceptors at the sympathetic nerve endings, is reduced in adriamycin-treated rabbits.

One explanation for the decreased noradrenaline release, through the activation of \( \beta \)-adrenoceptors at the sympathetic nerve endings in the adriamycin-treated rabbits, may involve myocardial cell damage, since adriamycin is known to have degenerative effects on cardiac myocytes.\textsuperscript{14,15}

Another possible explanation is that the decreased response of noradrenaline release is related to a decreased number, decreased affinity, and/or impaired signal transduction of presynaptic \( \beta \)-adrenoceptors caused by increased plasma noradrenaline in congestive heart failure. There has been no report on the number, affinity, or signal transduction of presynaptic \( \beta \)-adrenoceptors in rabbits with adriamycin-induced cardiomyopathy. In heart failure rabbits treated with adriamycin, however, there is controversy concerning the down-regulation of cardiac \( \beta \)-adrenoceptors. Some investigators have reported no down-regulation of cardiac \( \beta \)-adrenoceptors and no reduced production of cyclic AMP in response to isoproterenol in rabbits with adriamycin-induced heart failure while others have reported that there is down-regulation of \( \beta \)-adrenoceptors and reduced cyclic AMP production. The reason for the discrepancy may relate to the different doses of adriamycin used (1 mg/kg twice a week\textsuperscript{6} vs 0.7 mg/kg three times a week\textsuperscript{7} and the different durations of adriamycin treatment (8 weeks\textsuperscript{6} vs 11 weeks)). Cardiomyopathy was produced in the present study using adriamycin twice a week for 8 weeks, at a dose of 1 mg/kg.

If adriamycin affects presynaptic \( \beta \)-adrenoceptors and postsynaptic \( \beta \)-adrenoceptors in the same way, the adriamycin treatment used in the present study would not affect the number of presynaptic \( \beta \)-adrenoceptors or the production of cyclic AMP at the
sympathetic nerve endings. However, in view of our findings that noradrenaline release via the activation of presynaptic \( \beta \)-adrenoceptors decreased in rabbits with adriamycin-induced cardiomyopathy, adriamycin may effect the presynaptic \( \beta \)-adrenoceptors at the sympathetic nerve endings differently than it effects the postsynaptic \( \beta \)-adrenoceptors in the myocardium. It is possible that either a decreased number, decreased affinity or impaired signal transduction of presynaptic \( \beta \)-adrenoceptors was induced either by the elevated plasma noradrenaline due to cardiac failure or by some direct effect of adriamycin.

A previous study\(^{10}\) found that the function of presynaptic \( \alpha_2 \)-adrenoceptors was not impaired in rabbits with cardiomyopathy induced by the same adriamycin regimen used in the present study. It is therefore likely that this dose of adriamycin does not affect the sympathetic nerve endings via a direct toxic effect of adriamycin. While it is also possible that a toxic effect of adriamycin damaged the presynaptic \( \beta \)-adrenoceptors, decreased noradrenaline release from the sympathetic nerve endings in response to adrenaline might be due to down-regulation of the presynaptic \( \beta \)-adrenoceptors. This may be the case, since the plasma level of noradrenaline was elevated in rabbits with adriamycin-induced cardiomyopathy (Table I), and since \( \beta \)-adrenoceptors are reportedly easier to down-regulate than are \( \alpha_2 \)-adrenoceptors in patients with congestive heart failure\(^{16}\).

Presently, it is very difficult, in terms of methodology, to assess the affinity of presynaptic \( \beta \)-adrenoceptors. Therefore, we cannot exclude the possibility that the reduced affinity of presynaptic \( \beta \)-adrenoceptors due to elevated plasma noradrenaline contributed to a decrease in noradrenaline release in the adriamycin-treated rabbits in the present study. Impaired signal transduction of presynaptic \( \beta \)-adrenoceptors due to elevated plasma noradrenaline may also contribute to the observed decrease in noradrenaline release in response to adrenaline. It remains to be investigated whether the increased plasma noradrenaline caused decreased number, decreased affinity or impaired signal transductions of the presynaptic \( \beta \)-adrenoceptors.

In conclusion, the present study suggests that in rabbits with adriamycin-induced cardiomyopathy, the noradrenaline release from the sympathetic nerve endings via the activation of presynaptic \( \beta \)-adrenoceptors is reduced. This might be due to down-regulation of presynaptic \( \beta \)-adrenoceptors caused by the elevated plasma noradrenaline due to cardiac failure. However, other possibilities, such as reduced affinity or impaired signal transduction, cannot be excluded.

REFERENCES

8. STARKE K: Regulation of noradrenaline release by presynaptic receptor systems. Rev Physiol Biochem Pharmacol 1977; 77: 1—24


