Alterations in Vascular Sensitivity to Vasoactive Agents after Discontinuation of Propranolol in SHR

TETSUHIRO KUBOTA, M.S., AND TAKASHI YAMADA, M.D., PH.D.

SUMMARY Spontaneously hypertensive rats (SHR) were treated with propranolol (P) (70 mg/kg) daily for 2 or 4 weeks and then the effects of vasoactive substances on blood pressure were studied 10 or 36 hours after the last dose of P. At 10 hours after the last dose of P, vascular hyperresponsiveness to norepinephrine (NE) and angiotensin II (AII) had largely disappeared, but the hypotensive action of isoproterenol and prostacyclin was still blocked in P-treated SHR. An increase of cyclic AMP (cAMP) in response to isoproterenol was blocked in the thoracic aorta. Similarly, an increase of circulating cAMP and blood glucose in response to epinephrine (E) was depressed. At 36 hours after the last dose of P, an elevation of blood pressure in response to NE and AII was significantly reduced in P-treated SHR. Although basal blood pressure with or without anesthesia was the same in P-treated SHR and control SHR, a decrease of blood pressure in response to isoproterenol and prostacyclin was augmented significantly in P-treated SHR. This was also true in normal rats similarly treated. In addition, an increase of cAMP in the thoracic aorta in response to isoproterenol and prostacyclin was augmented significantly in P-treated SHR. An increase in blood glucose in response to E was not blocked, but an increase of circulating cAMP in response to E was blocked. These data suggest that cAMP synthesis in the vessels is somehow related to the production of peculiar vascular responses during escape from P action. It is also suggested that escape from P action is faster in the vessels and liver than in other tissues. (Hypertension 6: 249-254, 1984)

KEY WORDS • propranolol • rebound of blood pressure • vasoactive substance

BENEFICIAL effects of propranolol (P) in the treatment of hypertension are well established in humans. Some data have indicated, however, that P produces hypertension, particularly in patients with high circulating catecholamines.

Our previous study indicated that chronic administration of P potentiated the vasoconstrictive action of norepinephrine (NE) and angiotensin II (AII) in spontaneously hypertensive rats (SHR). In addition, the chronic administration of P inhibited the hypotensive action of isoproterenol and prostacyclin in SHR. Since each drug manifests its action through its specific receptor, it seems that chronic P administration modifies the action of vasoactive compounds by nonspecifically affecting multiple receptors or postreceptor sites of the vessels.

Interestingly, a rebound phenomenon has been noticed in the blood coagulation of the coronary artery after the abrupt discontinuation of P treatment, although such a rebound has not been reported in basal blood pressure. One may expect, however, that rebound vascular responsiveness to a number of vasodilating and vasoconstricting compounds can be produced after the abrupt discontinuation of P. To test this hypothesis, the present study was carried out in SHR and in normal rats.

Materials and Methods

Experiment 1

In 72 male SHR, P (70 mg/kg) or placebo (0.5% carboxymethyl cellulose) was administered orally daily for 2 or 4 weeks, beginning 10 weeks after the birth. The animals were used for the experiment 10 hours after the last dose of P. Under urethane anesthesia, arterial blood pressure and heart rate were continuously recorded from a carotid artery by a polyethylene catheter inserted into the artery and connected to a transducer. Graded doses of test materials (NE, AII, isoproterenol, and prostacyclin), 0.1 ml/100 g body weight, were injected into the femoral vein with an interval of 30 minutes. The NE, AII, isoproterenol, and prostacyclin-Na (PGI₂) were dissolved in physiologic saline to obtain the desired concentration.
Experiment 2
In 80 male SHR and 11 normal male Wistar Kyoto rats, P (70 mg/kg) or placebo was administered orally daily for 1 to 2 weeks, beginning 10 weeks after birth. At 36 hours after the last dose of P, with the animals under urethane anesthesia, changes in blood pressure in response to test substances (NE, All, isoproterenol, and PGI₂) were recorded as stated in Experiment 1. The basal blood pressure was also measured by the tail cuff method in unanesthetized rats.

Experiment 3
As stated in Experiments 1 and 2, 58 male SHR were treated with P (70 mg/kg) or placebo for 2 weeks. At 10 or 36 hours after the last dose of P, blood pressure was measured by the tail cuff method in unanesthetized rats. Then the rats were decapitated, the heart and thoracic aorta removed, washed three times with physiologic saline, and used to measure the basal concentration of cyclic AMP (cAMP). The washed thoracic aorta was then incubated with PGI₂ or isoproterenol for 15 minutes. The cAMP concentration was measured by radioimmunoassay as reported previously.

Experiment 4
As in earlier experiments, 60 male SHR were treated with P (70 mg/kg) or placebo for 2 weeks. At 10 or 36 hours after the last dose of P, 1 mg/kg of epinephrine (E) was administered intraperitoneally, and blood samples were obtained before and after (30 and 60 minutes) the E administration to measure blood glucose and plasma cAMP concentrations. Blood glucose was measured by autoanalyzer, while plasma cAMP concentration was determined by radioimmunoassay, as stated in Experiment 3.

Student's t test was used for statistical analysis. A p value less than 0.05 was considered statistically significant.

Results
Experiment 1. Blood Pressure Changes 10 Hours after Discontinuation of Propranolol Treatment in SHR
In the first step, the animals were treated orally with P (70 mg/kg) daily for 2 or 4 weeks, and then were used for the experiment 10 hours after the last dose of P. As compared to the controls, blood pressure under anesthesia was reduced significantly in the 4-week group (control = 186 ± 6 mm Hg; P = 162 ± 6 mm Hg; p < 0.05) but not significantly so in the 2-week group (control = 168 ± 5 mm Hg; P = 154 ± 6 mm Hg). As shown in Figure 1 left, a net increase in blood pressure in response to NE was the same in P-treated SHR and in the controls when the animals were treated with P for 2 weeks. In contrast, a net increase in blood pressure in response to NE was more in P-treated SHR than in the controls when the animals were treated with P for 4 weeks.

In the second step, a similar type of experiment was performed, using All as the vasoactive substance (Figure 1 right). The blood pressure in anesthetized rats was comparable to that found in the first step of the experiment. The net blood pressure increase in response to All was the same in P-treated SHR and controls when P was administered for 2 weeks. The increase was slightly but insignificantly more in P-treated SHR than controls when P treatment was 4 weeks.

In the third step, the animals were treated with P (70 mg/kg) for 2 weeks and used for the experiment 10 hours after the last dose of P. The blood pressure in anesthetized rats was comparable to that found in the first step of the experiment. Administration of isoproterenol produced a dose-related decrease in blood pressure in the controls but failed to do so in P-treated SHR (Figure 2 left). Rather, a large dose of isoproterenol raised blood pressure in P-treated SHR.

Finally, a similar type of experiment was performed, using PGI₂ as the vasoactive substance (Figure...
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FIGURE 2. Effects of graded doses of isoproterenol and prostacyclin on blood pressure in spontaneously hypertensive rats (SHR) 10 hours after the last dose of propranolol, which was administered for 2 weeks. Bars and vertical lines indicated means ± SE calculated from six animals.

Experiment 2. Blood Pressure Changes 36 Hours after the Last Dose of Propranolol

In the first step of the experiment, the animals were treated daily with oral P (70 mg/kg) for 2 weeks and used for the experiment 36 hours after the last dose of P. At this time, blood pressure returned to the control levels under anesthetized and unanesthetized conditions. As shown in Figure 3 left, a net increase in blood pressure in response to NE was less in P-treated SHR than in the controls. The difference was statistically significant when 3.0 μg/kg NE was used. A net increase in blood pressure in response to All was also less in P-treated SHR than in the controls (Fig. 3 right). When 0.1 μg/kg All was used, the difference was statistically significant.

FIGURE 3. Effects of norepinephrine and angiotensin II on blood pressure in SHR 36 hours after the last dose of propranolol, which was administered for 2 weeks. Bars and vertical lines indicated means ± se calculated from six animals.
In the second step, the animals were treated with P (70 mg/kg) for 1, 7, and 14 days, and the animals were used 36 hours after the last dose of P. At this time, the blood pressure in rats under anesthesia was comparable to that found in controls. As shown in Table 1, dose-related decreases in blood pressure were produced by the administration of graded doses of isoproterenol and PGI\textsubscript{2}. The decrease was the same in the controls and P-treated SHR when P was administered for 1 or 7 days. However, the decrease was significantly more in P-treated SHR than in the controls when P was administered for 2 weeks.

Finally, the effects of isoproterenol and PGI\textsubscript{2} on blood pressure were studied in normal rats similarly treated (Table 1). In anesthetized rats, blood pressure was 95.0 ± 2.94 mm Hg in the controls and 99.0 ± 4.79 mm Hg in the P-treated rats. Administration of isoproterenol and PGI\textsubscript{2} reduced blood pressure in a dose-related manner. The net decrease was significantly more in P-treated SHR than in the controls when P was administered for 2 weeks.

Experiment 3. Cyclic AMP Concentration in the Heart and Thoracic Aorta after Discontinuation of Propranolol

In the first step, the animals were treated with oral P (70 mg/kg) daily for 2 or 4 weeks. At 10 hours after the last dose of P, the animals were used for the experiment. When measured by the tail cuff method while conscious, SHR treated with P for 4 weeks showed a significant reduction in blood pressure, but the SHR treated with P for 2 weeks did not (Table 2). The basal concentration of cAMP in the heart and thoracic aorta was reduced slightly in P-treated SHR, but the difference was not statistically significant. When the thoracic aorta was incubated with isoproterenol (10\textsuperscript{-6} g/ml), a significant cAMP increase was found. The increase was significantly less in P-treated SHR than in the controls, however.

In the second step, the animals were treated with P (70 mg/kg) for 2 weeks, and the animals were used for the experiment 36 hours after the last dose of P. Blood pressure in P-treated SHR returned to untreated levels when the blood pressure was measured by the tail cuff method in conscious rats. After incubation of the thoracic aorta with PGI\textsubscript{2}, an increase of cAMP concentra-
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**TABLE 3. Increase of Cyclic AMP in the Thoracic Aorta in Response to Prostacyclin and Isoproterenol 36 Hours after Discontinuation of Propranolol (P) Treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Cyclic AMP concentration (pmol/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5% CMC for 2 wks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70 mg/kg P for 2 wks</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.34 ± 0.047</td>
</tr>
<tr>
<td>Prostacyclin 10−6 g/ml</td>
<td>6</td>
<td>0.40 ± 0.017</td>
</tr>
<tr>
<td>Prostacyclin 10−7 g/ml</td>
<td>6</td>
<td>0.35 ± 0.054</td>
</tr>
<tr>
<td>Isoproterenol 10−5 g/ml</td>
<td>6</td>
<td>0.80 ± 0.149</td>
</tr>
<tr>
<td>Isoproterenol 10−6 g/ml</td>
<td>6</td>
<td>0.46 ± 0.032  *</td>
</tr>
</tbody>
</table>

Values are means ± se. CMC = carboxymethyl cellulose.

* p < 0.05 as compared to 0.5% CMC control.

A significant increase was found in the thoracic aorta. The increase was significantly more in P-treated SHR than in controls when a large dose of PGI2 was used (Table 3). Also, an increase of cAMP in response to isoproterenol was found in P-treated SHR and in controls. The increase was significantly more in P-treated SHR than in the controls when 10−6 g/ml isoproterenol was used.

**Experiment 4. Circulating Cyclic AMP and Blood Glucose in Response to Epinephrine after Discontinuation of Propranolol**

In the first step, the animals were treated with P (70 mg/kg) for 2 weeks. When the animals were used 10 hours after the last dose of P, the blood pressure of conscious rats was comparable to that found in Table 2. The basal concentration of plasma cAMP was the same in the controls and in P-treated SHR (Table 4). Plasma cAMP concentration increased significantly 30 minutes after administration of E in the controls but not in the P-treated SHR. At 36 hours after discontinuation of P, the basal plasma cAMP concentration was again the same in P-treated SHR and in the controls. An increase of plasma cAMP was found 30 minutes after the administration of E in the controls but not in the P-treated SHR.

In the second step, the animals were treated daily with P (70 mg/kg) for 2 weeks. The blood pressure in conscious rats 10 or 36 hours after the last dose of P was comparable to that found in Table 2. When the animals were used for the experiment 10 hours after the last dose of P, blood glucose increased significantly after administration of E, but the increase was significantly less in P-treated SHR than in the controls. No difference in an increase of blood glucose was found between the controls and P-treated SHR 36 hours after the last dose of P (Table 5).

**TABLE 4. Increase of Circulating cAMP in Response to Epinephrine after Discontinuation of Propranolol (P)**

<table>
<thead>
<tr>
<th>Group</th>
<th>P dose (mg/kg)</th>
<th>No. of rats</th>
<th>Circulating cAMP after injection of epinephrine (1 mg/kg i.p.) pmol/ml of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 10 hours</td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control (0.5% CMC)</td>
<td>—</td>
<td>5</td>
<td>58.9 ± 1.23</td>
</tr>
<tr>
<td>P</td>
<td>70</td>
<td>6</td>
<td>61.5 ± 1.39</td>
</tr>
<tr>
<td>After 36 hours</td>
<td></td>
<td></td>
<td>54.9 ± 4.97</td>
</tr>
<tr>
<td>Control (0.5% CMC)</td>
<td>—</td>
<td>8</td>
<td>53.4 ± 10.49</td>
</tr>
<tr>
<td>P</td>
<td>70</td>
<td>10</td>
<td>61.2 ± 10.49</td>
</tr>
</tbody>
</table>

Values are means ± se.

* p < 0.01.
† p < 0.005.

**TABLE 5. Increase of Blood Glucose in Response to Epinephrine after Discontinuation of Propranolol (P) Treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>P dose (mg/kg)</th>
<th>No. of rats</th>
<th>Blood glucose after injection of epinephrine (1 mg/kg i.p.) mg/100 ml of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 10 hours</td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control (0.5% CMC)</td>
<td>—</td>
<td>5</td>
<td>148.4 ± 6.42</td>
</tr>
<tr>
<td>P</td>
<td>70</td>
<td>6</td>
<td>146.8 ± 3.82</td>
</tr>
<tr>
<td>After 36 hours</td>
<td></td>
<td></td>
<td>142.5 ± 4.62</td>
</tr>
<tr>
<td>Control (0.5% CMC)</td>
<td>—</td>
<td>9</td>
<td>134.8 ± 6.36</td>
</tr>
<tr>
<td>P</td>
<td>70</td>
<td>11</td>
<td>134.8 ± 6.36</td>
</tr>
</tbody>
</table>

Values are means ± se.

* p < 0.005 as compared to the control.
Discussion

Our previous study indicated that in vivo and in vitro responses of blood vessels to NE and All were augmented during chronic treatment with P in SHR, and that hypotensive action of isoproterenol and PGI₂ was blocked under a similar experimental condition. It seems, therefore, that although the hypertension was eradicated by P, the vessels produced hypertension in response to vasoactive substances. One may assume, then, that such an untoward action of P will disappear after discontinuation of P treatment. To test this hypothesis, we studied vascular responses to vasoactive substances 10 or 36 hours after discontinuation of P in SHR.

At 10 hours after the last dose of P, the blood pressure was slightly or significantly below control level, depending on the duration of the P treatment. Vascular hyperresponsiveness to NE and All largely disappeared, but hypotensive action of isoproterenol and PGI₂ was still blocked. Thus, there was a discrepancy between vasoconstrictive and vasodilating responses during escape from P action. Since cAMP has been somehow related to the dilation of vessels, the cAMP concentration was studied 10 hours after the last dose of P. Although the basal concentration of cAMP in the heart and thoracic aorta was the same in the controls and P-treated SHR 10 hours after P treatment, an increase in cAMP in the thoracic aorta in response to isoproterenol was blocked in P-treated SHR. Thus, a decrease of relaxation of vessels was well correlated with a decrease of cAMP synthesis when isoproterenol was used as the stimulator. However, such a block of cAMP synthesis is not specific for blood vessels, but is common in other tissues, since an increase of circulating cAMP and blood glucose in response to E was depressed significantly under this experimental condition.

At 36 hours after discontinuation of P, the blood pressure was comparable to that found in the controls in anesthetized and conscious rats. However, a number of interesting aspects were found. First, an increase in blood pressure in response to NE and All was reduced significantly in P-treated SHR. Second, a decrease in blood pressure in response to isoproterenol and PGI₂ was augmented significantly when SHR were chronically treated with P. This was also true when normal rats were chronically treated with P. Third, such an augmentation of PGI₂ and isoproterenol action was not found when SHR were treated with P for a short period. Fourth, an increase in cAMP in the thoracic aorta in response to isoproterenol and PGI₂ was augmented significantly in SHR chronically treated with P. It seems, therefore, that during escape from P action there is a rebound phenomenon in vascular responses and cAMP synthesis in response to vasoactive substances. The exact mechanism through which the rebound is produced is not known at present.

Whatever the exact explanation for the rebound phenomenon may be, it was found that the increased blood glucose in response to E was normal 36 hours afterward. Since this glucose increase is possibly mediated through an increase of hepatic cAMP, it seems that cAMP synthesis by the liver was normal 36 hours after discontinuation of P. On the other hand, an increase of circulating cAMP in response to E was still depressed 36 hours after P. This indicated that escape from P action is faster in the vessels and liver than in most other tissues or organs.

References

10. Sutherland EW, Robison GA. The role of cyclic AMP in the control of carbohydrate metabolism. Diabetes 1969;18:797-819
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