Neuropeptide Y Prevents the Blood Pressure Fall Induced by Endotoxin in Conscious Rats With Adrenal Medullectomy

Dominique Evéquoz, Bernard Waeber, Jean-François Aubert, Jean-Pierre Flückiger, Jürg Nussberger, and Hans R. Brunner

Neuropeptide Y (NPY) is a vasoconstrictor peptide known to be present in the adrenal medulla, the terminal nerve endings, and in plasma. This study was designed to test whether NPY could prevent the acute blood pressure fall induced by endotoxin administration. Normotensive rats were subjected to adrenal demedullation on the right side and were either adrenalectomized or sham-operated on the left side. Eight to ten days later, NPY (0.07 μg/min i.v.) or its vehicle were infused for 95 minutes into these conscious, semirestrained rats. The same experiments were performed with rats that received an infusion of epinephrine (0.1 μg/min). These doses of NPY and epinephrine when given alone had no blood pressure effect. During the last 75 minutes of the 95-minute infusion, endotoxin (lipopolysaccharide Escherichia coli 0.111:B4, 10 μg/min i.v.) or its vehicle were administered. In rats with an intact adrenal gland, endotoxin failed to induce hypotension. In rats lacking a functioning adrenal medulla, however, endotoxin induced a pronounced mean blood pressure fall of 55 ± 11.6 mm Hg (mean ± SEM). This blood pressure drop could be prevented equally well with NPY and with epinephrine infusion and averaged 11 ± 2.3 and 16 ± 2.4 mm Hg, respectively, at the end of the experiment. Additional rats were biadrenalectomized and supplemented with an excess of glucocorticoids and mineralocorticoids. In these rats also, NPY markedly attenuated the blood pressure fall resulting from endotoxemia. These data taken together indicate that in conscious rats with no adrenal medulla, the acute blood pressure fall induced by endotoxin administration is greatly enhanced. In these rats, a nonpressor dose of NPY prevents the hypotensive effect of endotoxin equally well as an epinephrine infusion. (Circulation Research 1988;62:25-30)
removed and, on the right side, the four suprarenal nerves were cut.

Eight to ten days after the initial surgery, under light ether anesthesia, the right external iliac artery of all animals was cannulated with a PE-50 catheter. A PE-10 catheter was introduced in both the right femoral vein and the right jugular vein. All catheters contained a heparinized 0.9% NaCl solution (300 U/ml) and were exteriorized on the back of the animal. The rats were then placed in a plastic tube specially designed for partial restriction of their movements and were left there to wake up. Thereafter, the animals appeared to rest comfortably throughout the duration of the experiment. Arterial pressure and heart rate were monitored via a pressure transducer (Statham, Hato Rey, Puerto Rico) connected to an electrogalvanometer (Philips 2600, Eindhoven, The Netherlands) and recorded on a light-sensitive oscillograph (Mannarp 150, Electronic Institute, London). The experiment was started 90 to 120 minutes after the end of anesthesia, when blood pressure and heart rate were stable.

The different study groups are outlined in Table 1. Group 1 animals underwent a sham-adrenalectomy on the left side and had the right adrenal gland denervated. Group 2 animals were adrenalectomized on the left side and had the right adrenal gland denervated. Group 3 animals served as controls and received a 95-minute infusion of NPY (0.07 μg/min i.v.). In Group 4, the second infusion contained, instead, the vehicle of NPY, and in Group 5, the first infusion contained NPY and the second contained endotoxin. In Group 6, the first infusion contained NPY (0.07 μg/min i.v.) and the second contained endotoxin. Group 7 received NPY and then endotoxin, and finally, Group 8 received epinephrine and NPY in the 95-minute infusion and endotoxin in the 75-minute infusion. The rats of Group 9 subjected to adrenal denervation were infused with the NPY vehicle and endotoxin. The different solutions were administered using a syringe pump (model 455, Sage Instrument, White Plains, N.Y.). At the end of the observation period, a 0.3-ml blood sample was collected in all rats through the arterial catheter for determination of plasma catecholamine levels using a radioenzymatic method. Immediately thereafter, all animals were killed using an overdose of barbiturate.

### Table 1. Characteristics of the Study Groups of Part 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MBP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Endotoxin (μg/min)</th>
<th>NPY (μg/min)</th>
<th>Epinephrine (μg/min)</th>
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<tbody>
<tr>
<td>Sham</td>
<td>1</td>
<td>10</td>
<td>118 ± 2.5</td>
<td>424 ± 14</td>
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<td>-</td>
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<tr>
<td>Med</td>
<td>2</td>
<td>8</td>
<td>116 ± 2.7</td>
<td>458 ± 22</td>
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<tr>
<td></td>
<td>3</td>
<td>11</td>
<td>116 ± 2.5</td>
<td>406 ± 18</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td>4</td>
<td>9</td>
<td>111 ± 3.6</td>
<td>438 ± 19</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>5</td>
<td>12</td>
<td>116 ± 1.6</td>
<td>434 ± 10</td>
<td>+</td>
<td>-</td>
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<td></td>
<td>6</td>
<td>6</td>
<td>117 ± 2.1</td>
<td>384 ± 16</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7</td>
<td>118 ± 2</td>
<td>393 ± 26</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>8</td>
<td>11</td>
<td>118 ± 4</td>
<td>429 ± 19</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Den</td>
<td>9</td>
<td>5</td>
<td>124 ± 4</td>
<td>394 ± 23</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM.*

*+, Groups with procedure done; -, groups without procedure.

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**Part 2**

The rats of Group 10 were sham-operated, and those of Groups 11 to 14 were biadrenalectomized under ether anesthesia. They were all injected subcutaneously with methylprednisolone acetate, 20 mg/kg (Depomedrol, 40 mg/ml, The Upjohn Co., Kalamazoo, Mich.) and deoxytocicosterone pivalate, 10 mg/kg (Percorten-M, 25 mg/ml, Ciba-Geigy). The animals were then returned to their cages and the experiments were performed 2 days later. On the day of the experiment, the animals were prepared as described in Part 1. They were infused for 95 minutes with either NPY, 0.07 μg/min i.v. (Groups 12 and 14), or its vehicle (Groups 11 and 13) (Table 2). Endotoxin, 0.07 μg/min i.v., was administered during the last 75 minutes in rats of Groups 10, 13, and 14. The other rats (Groups 11 and 12) received the vehicle of endotoxin during the corresponding period.
Table 2. Characteristics of the Study Groups of Part 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Baseline</th>
<th>Endotoxin</th>
<th>NPY NPY0.07</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MBP mm Hg</td>
<td>HR beats/min</td>
<td>10 µg/min</td>
</tr>
<tr>
<td>Sham</td>
<td>10</td>
<td>127±4.5</td>
<td>374±20</td>
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<td>Adr</td>
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<td>137±3.8</td>
<td>406±27</td>
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<tr>
<td></td>
<td>14</td>
<td>131±1.7</td>
<td>398±11</td>
<td>+</td>
</tr>
</tbody>
</table>

Adr, adrenalectomized; other abbreviations the same as Table 1. + , Groups with procedure done; – , groups without procedure. Values are mean ± SEM.

Chemicals

Neuropeptide Y (Sigma Chemical Company, St. Louis, Mo.) was dissolved in 0.9% NaCl to achieve a concentration of 7 mg/1. Aliquots containing 10 µg were kept frozen at −70° C. They were thawed immediately before use on the day of the experiment. Epinephrine (Sigma) was also diluted in 0.9% NaCl to a final concentration of 10 mg/1. For the rats treated simultaneously with NPY and epinephrine, the concentration of both agents was doubled. Equal volumes of the two solutions were then mixed so that there were 0.07 µg NPY and 0.1 µg epinephrine in 10 µl of this preparation.

Data are reported as mean ± SEM. Statistical evaluation of the results was made using a one-way analysis of variance. When the $p$ value was less than 0.05, multiple comparisons were performed using a Fisher’s least significant test.

Results

Part 1

There was no significant difference in body weight, baseline intra-arterial mean blood pressure, and heart rate between the different groups of rats.

Figure 1 shows the effect of endotoxin on mean blood pressure and heart rate of rats having one adrenal intact (Group 1). In these animals, the trend of mean blood pressure to decrease during endotoxin infusion achieved a significant level only at the end of the experiment, when it had fallen from 118±2.5 to 107±2.3 mm Hg ($p<0.01$). No significant change in heart rate was observed.

In Figure 2, the results obtained in medullectomized rats infused with NPY (Group 3) or its vehicle (Group 2) are described. These rats were also given the vehicle of endotoxin. NPY changed neither mean blood pressure nor heart rate.

Endotoxin given alone induced a progressive and pronounced fall in mean blood pressure from 111±3.6 to 73±7.6 mm Hg ($p<0.001$) in medullectomized rats (Group 4, Figure 3). The results illustrated here represent those obtained in 7 of the 9 rats included in this group. The two other animals died before the end of the study. In NPY-treated rats (Group 5), the endotoxin-induced blood reduction was almost completely prevented since it fell only from 116±1.6 to 107±2.9 mm Hg. Heart rate increased in both groups of rats. The acceleration was significant only in animals given NPY.
Epinephrine had no effect on mean blood pressure or heart rate of the medullectomized rats infused with the vehicle of endotoxin (Group 6, Figure 4). When endotoxin was added to epinephrine (Group 7), mean blood pressure was reduced from 118 ± 2 to only 103 ± 2.3 mm Hg (p < 0.01). In these rats, too, endotoxin administration significantly raised heart rate. In rats infused simultaneously with NPY and epinephrine (Group 8), endotoxin had no significant effect on mean blood pressure or heart rate (Figure 5).

Figure 6 depicts the plasma levels of epinephrine (upper panel) and norepinephrine (lower panel) determined at the end of the study in 8 different groups of rats. Epinephrine was not detectable in medullectomized rats unless exogenous epinephrine was administered. Plasma epinephrine levels were significantly higher in sham-operated rats (3.11 ± 0.55 ng/ml, Group 1) than in medullectomized rats infused with epinephrine alone (1.04 ± 0.25 ng/ml, Group 6, p < 0.05) or in combination with endotoxin (2.07 ± 0.53, Group 7, p < 0.05). They were, however, similar in medullectomized rats receiving endotoxin and treated with NPY and epinephrine together (3.32 ± 0.54 ng/ml, Group 8). Plasma norepinephrine levels were higher (not significantly) in medullectomized rats infused with endotoxin alone (3.80 ± 1.0 ng/ml, Group 4) than in sham-operated controls (2.34 ± 0.5 ng/ml, Group 1). There was also no significant difference in plasma norepinephrine concentrations between the latter rats and medullectomized rats infused with endotoxin combined with NPY and/or epinephrine.

After adrenal denervation (Group 9), endotoxin decreased mean blood pressure from 124 ± 4 to 116 ± 7.3 mm Hg. Heart rate averaged 394 ± 23 beats/min before starting endotoxin infusion and 430 ± 16 beats/min at the end of the experiment. Circulating epinephrine levels were lower (0.64 ± 0.3 ng/ml) than in endotoxin-treated rats having a normally innervated adrenal (Group 1, 3.11 ± 0.55 ng/ml, p < 0.05). There was also a trend for plasma norepinephrine to be lower in denervated rats (1.33 ± 0.3 ng/ml) than in sham-operated animals (2.34 ± 0.51 ng/ml).

Part 2
Endotoxin markedly reduced mean blood pressure (from 130 ± 3.5 to 90 ± 13 mm Hg, p < 0.001, Figure 7) in adrenalectomized rats pretreated with exogenous glucocorticoids and mineralocorticoids (Group 13).
rats infused with NPY (Group 14), the endotoxin-induced reduction in mean blood pressure was far less pronounced although still significant since it fell from 131±1.7 to 118±4.3 mm Hg. In corresponding control rats (Groups 11 and 12), mean blood pressure was not significantly altered during the experiment. Endotoxin had no significant effect on heart rate neither in control nor in adrenalectomized rats. In sham-operated rats, endotoxin had no blood pressure effect (Group 10).

Discussion

The lethal effect of endotoxin in rats is known to be greatly enhanced by adrenalectomy.21,22 Administration of exogenous glucocorticoids counteracts only in part the marked hypotensive effect produced by endotoxin in adrenalectomized rats.21 These hormones originating from the adrenal cortex are thought to protect against endotoxin by preventing cachectin release from macrophages.23 They have to be given together with epinephrine to restore vascular resistance to endotoxin after removal of the adrenals.21 The results of the present series of experiments in rats with adrenal medullectomy are in agreement with the observations made previously.22 Thus, our rats, lacking a normally functioning adrenal medulla, developed severe hypotension when exposed to endotoxin, whereas in animals with intact adrenals, the same dose of endotoxin had no major blood pressure lowering effect. As expected, the epinephrine infusion was beneficial in our medullectomized rats since it prevented to a large extent the occurrence of the endotoxin-induced fall in blood pressure.

In our rats subjected to adrenal medullectomy, the production of endogenous steroids was presumably sufficient to avoid the development of adrenal cortical insufficiency. Thus, 8 to 10 days after surgery, the rats looked healthy. More important, their baseline blood pressure was not lower than that of sham-operated controls, a finding that strongly argues against the presence of adrenal cortical insufficiency.24 On the other hand, plasma norepinephrine levels of our medullectomized rats were found to be more than twice those we usually measure in normotensive rats with intact adrenals.25 This might reflect the presence of some degree of adrenal insufficiency. The concentration of circulating norepinephrine is indeed known to be elevated in hormone-deficient rats with mineralocorticoid activity.26 It is, however, also possible that the abnormally high plasma norepinephrine levels were due to an increased sympathoneural activity in response to the prolonged absence of epinephrine in the blood.

The blood pressure effect of endotoxin given alone or together with NPY was also assessed in adrenalectomized rats supplemented with an excess of glucocorticoids and mineralocorticoids. The doses of methylprednisolone used in the present study suffice to produce hypertension in rats when administered on a weekly basis.27 This certainly accounts for the fact that baseline blood pressure tended to be higher in glucocorticoid-treated adrenalectomized rats than in the medullectomized rats.

The most striking feature of the present study in medullectomized rats is provided by the marked beneficial effect of NPY on the acute hemodynamic response to endotoxemia. NPY, at least as well as epinephrine, rendered the medullectomized rats resistant to endotoxin. It turned out that the fall in blood pressure resulting from endotoxin infusion was of similar magnitude in control rats with an intact adrenal function and in medullectomized rats treated with NPY. These results were obtained with a dose of NPY chosen to exert no pressor action by itself. The same kind of observation was made in adrenalectomized rats treated with exogenous glucocorticoids and mineralocorticoids. In these rats, also, the endotoxin-induced hypotension could be largely prevented by the infusion of NPY.

NPY has been identified not only in the central nervous system, but also in the periphery, in the adrenal medulla as well as within nerve terminals.11-13 In addition, this peptide has been detected in plasma and it is thought to be released either from the adrenal medulla or the catecholamine containing nerve terminals.13-15 In these rats, also, the endotoxin-induced hypotension could be largely prevented by the infusion of NPY.

As expected from previous work, plasma norepinephrine and particularly epinephrine reached very high levels during endotoxemia in rats having an intact adrenal.25,26 This rise in plasma catecholamines is known to occur in rats even when endotoxin is administered at doses lacking a blood pressure lowering effect and can be diminished by pretreating the animals with a cyclooxygenase inhibitor.28 Not surprisingly,
epinephrine was not detectable in the circulation of medullectomized rats unless it was infused. It is of note in this respect that for the same rate of epinephrine infusion, plasma epinephrine concentrations were higher when endotoxin was given simultaneously with the pressor amine. One possible explanation is that endotoxin impaired the uptake of epinephrine by terminal nerve endings.1

Plasma norepinephrine levels were similarly elevated in all but one group of endotoxin-treated rats. Only rats that experienced major hypotension as a result of endotoxin exhibited clearly higher plasma norepinephrine concentrations. Accordingly, this further rise in plasma norepinephrine levels could be attributed to a hypotension-induced reflex activation of the sympathetic nervous system.

Endotoxin administration at the doses used in these experiments has been shown to raise plasma catecholamines in animals with intact adrenals.2 In the present study, adrenal denervation attenuated this endotoxin-induced rise. However, in these denervated rats, endotoxin failed to lower blood pressure significantly, possibly because NPY could be released from the intact adrenal medulla. In the light of these findings, it appears that endotoxin triggers the release of catecholamines from the adrenal medulla at least to some extent through a neural mechanism, even when it is administered at nonhypotensive doses. Nevertheless, it cannot be ruled out that endotoxin shunts blood flow away from organs playing predominant role in clearing catecholamines from plasma.

In conclusion, the findings of the present experiments performed in unanesthetized rats indicate that the acute blood pressure fall induced by endotoxin is greatly enhanced by adrenal medullectomy. They also demonstrate that in rats without a normally functioning adrenal medulla, nonpressor doses of NPY can prevent the endotoxin-induced blood pressure reduction just as well as, if not better than, epinephrine.

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References
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