Sympathoadrenal Control by Paraventricular Hypothalamic \(\beta\)-Endorphin in Hypertension

Changbae Jin and Robin William Rockhold

The paraventricular hypothalamus regulates autonomic nerve outflow and is innervated with \(\beta\)-endorphin-immunoreactive nerve terminals. This study examined the effects of \(\beta\)-endorphin microinjected into the paraventricular hypothalamus on blood pressure, heart rate, and plasma catecholamine and glucose concentrations in conscious, unrestrained spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats at the age of about 9 weeks. Thirty minutes after paraventricular hypothalamic injection of \([^{125}I]\)\(\beta\)-endorphin (3.5 \(\mu\)g), most of the recovered radioactivity was detectable within \(\pm\)0.5 mm from the injection site in the coronal, sagittal, and horizontal planes. Unilateral paraventricular hypothalamic injections of \(\beta\)-endorphin (1 and 0.1 \(\mu\)g/0.1 \(\mu\)l) increased blood pressure and heart rate in both strains in a dose-independent manner with significantly greater increases in SHR. Plasma catecholamine and glucose concentrations were measured 15, 30, and 60 minutes after \(\beta\)-endorphin injection. Norepinephrine concentrations were not significantly altered in WKY rats but increased in SHR. Epinephrine concentrations increased in both strains with significantly greater increases in SHR. Increases in catecholamine concentrations were not dose-related. Glucose concentrations also increased in both strains with significantly greater increases in SHR only at the lower dose. Ganglionic blockade with pentolinium significantly reduced \(\beta\)-endorphin-induced pressor and tachycardiac responses in SHR. Pretreatment of the paraventricular hypothalamus with naltrexone (1.1 \(\mu\)g) in SHR blocked the initial pressor and tachycardiac responses to \(\beta\)-endorphin (0.1 \(\mu\)g) and blunted increases in epinephrine and glucose levels. When the animals were anesthetized with \(\alpha\)-chloralose 2–5 days after the study in conscious animals, there were no differences in blood pressure or heart rate between strains after \(\beta\)-endorphin (0.1 \(\mu\)g) injection. The results indicate that conscious SHR show enhanced cardiovascular and sympathoadrenal responses to \(\beta\)-endorphin injected into the paraventricular hypothalamus, suggesting that alterations in the activity of the paraventricular hypothalamic \(\beta\)-endorphin system can modulate the development of hypertension in SHR. (Hypertension 1991;18:503–515)

In addition to the well-known projections of the paraventricular hypothalamus (PVH) to the neurohypophysis, anatomic and electrophysiological studies have shown that the PVH projects directly to autonomic centers in the medulla and spinal cord, indicating an involvement of the PVH in cardiovascular regulation.\(^1\)–\(^3\) Recently, elegant neuroanatomic studies have shown that parvocellular neurons of the PVH regulate the entire sympathetic outflow, including that to the adrenal gland, in a topographic fashion.\(^4\)–\(^5\) Electrical or excitatory amino acid–induced stimulation of the PVH has been shown to alter systemic and regional hemodynamics.\(^6\)–\(^11\) However, the cardiovascular responses to electrical or excitatory amino acid–induced stimulation of the PVH are still controversial in anesthetized animals. In contrast, in conscious rats, electrical or excitatory amino acid–induced stimulation of the PVH has been shown to produce a consistent increase in blood pressure.\(^8\)–\(^9\) Therefore, it is of great importance to use conscious animals in investigating the cardiovascular regulation by the PVH. In this investigation, both anesthetized and conscious animals were used to examine the cardiovascular regulation by the PVH \(\beta\)-endorphin system.

In contrast to stimulation studies of the PVH, electrolytic\(^12\) or excitotoxin-induced\(^13\) lesions of the PVH produced no effects on resting levels of arterial pressure and heart rate in conscious, normotensive rats. Moreover, the majority of spinally projecting parvocellular PVH neurons normally show little or no activity.
no spontaneous electrical activity in urethane-anesthetized rats. These results indicate that the PVH does not play a significant role in maintenance of resting cardiovascular function. Rather, neurons within the PVH are thought to integrate autonomic nervous activity with specific behaviors or neuroendocrine secretion. It has long been known that the PVH is critically involved in mediation of specific feeding behaviors. Acute feeding with highly palatable diets rich in sucrose has been shown to activate hypothalamic $\beta$-endorphin pools and sucrose feeding to stimulate central sympathetic outflow. The PVH is a major terminal field for $\beta$-endorphin-containing neurons, and administration of the $\mu$-opioid receptor agonist [d-Ala$^3$, N-Me-Phe$^4$, Gly$^5$-ol]-enkephalin (DAGO), into the PVH has been shown to produce dose-related stimulatory effects on systemic hemodynamics and sympathoadrenal outflow. Therefore, $\beta$-endorphin in the PVH has been hypothesized to be involved in the integrative function of the PVH through regulation of sympathoadrenal function in association with feeding. Both the PVH and central opioid systems have been shown to be involved in the development of hypertension in spontaneously hypertensive rats (SHR). For example, ablation of the PVH delayed the development of hypertension in SHR. In addition, the pressor responses to centrally administered opioid peptides were enhanced in SHR as compared with those in Wistar-Kyoto (WKY) rats. Moreover, manipulation of food intake has been demonstrated to produce differential effects on systemic cardiovascular function between SHR and WKY rats. Thus, in the present study, $\beta$-endorphin was microinjected into the PVH of SHR and WKY rats to elucidate a putative role for $\beta$-endorphin as a neurotransmitter in the hypothalamic $\beta$-endorphin pools involved in the PVH through regulation of sympathoadrenal function by PVH neurons and a role of this system in the development of hypertension in SHR.

Methods

Male, 6-week-old SHR and WKY rats were purchased from Taconic Farms, Inc., Germantown, N.Y. The rats were housed, before use, in plastic group cages (3-4/cage), under controlled conditions of temperature (22-24°C), humidity (50-60%), and light/dark cycles (12-hour) and were maintained ad libitum on Rodent Laboratory Chow (Purina Mills, Inc., St. Louis, Mo.) and tap water. The rats were allowed 3-4 days for adaptation before the surgical procedures were performed.

The rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.; Nembutal, Abbott Laboratories, North Chicago, Ill.) and were mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif.). The surface of the skull was exposed and the head was oriented such that the skull sutures bregma and lambda were at the same vertical levels. A burr hole (2.8 mm) was drilled through the skull over a desired location. After carefully incising the dura and gently retracting the sagittal sinus, a stainless steel guide cannula (26 gauge) was implanted to terminate 1 mm above the left PVH (1.8 mm posterior for WKY rats, 1.6 mm posterior for SHR, 0.4 mm left from bregma, and 6.7 mm below the surface of the dura). This cannula was secured to the skull with stainless steel machine screws and dental acrylic and then plugged with a stainless steel stylet. Following a recovery period of 9-10 days after cannulation of the PVH, polyethylene catheters (PE-50, Clay Adams, Parsippany, N.J.) filled with heparinized saline (100 units/ml) were implanted into the abdominal aorta and inferior vena cava by way of femoral artery and vein, respectively, while the rats were under halothane anesthesia. These catheters were exteriorized at the nape of the neck and sealed by heating the tips. The rats were allowed to recover for at least 2 days while being housed individually in hanging wire cages. After each surgical procedure, the rats were given a single injection of procaine penicillin G (60,000 units s.c.; Pfizerpen, Roerig, New York). After PVH cannulation, during the recovery period before actual experiments, the rats were acclimated to handling procedures. These included mock PVH injections and daily placement in a Plexiglas experimental cage (10 in. long x 3.5 in. wide x 3 in. deep), which restricted range of motion but left them unrestrained.

At the time of the experiment, the rats (8-9 weeks old) were placed in experimental cages. The arterial catheter was connected to a pressure transducer (Cobe Laboratories, Inc., Lakewood, Colo.) and a polygraph (model 7D, Grass Instrument Co., Quincy, Mass.) for monitoring arterial pressure and heart rate. When animals were resting quietly and systemic cardiovascular function had stabilized, approximately 1.3 ml arterial blood was drawn into an ice-chilled, heparinized syringe for about 5 minutes to measure resting levels of plasma catecholamines and glucose while intravenous transfusion of donor blood was simultaneously performed at the same rate using an infusion/withdrawal pump (model 600-950, Harvard Apparatus, Inc., South Natick, Mass.). The control blood sample was immediately centrifuged in a cold room (4°C) to separate plasma. A 33-gauge stainless steel injector was lowered into the PVH through the guide cannula after removing the stylet. The length of the injector was measured precisely to extend 1 mm beyond the tip of the guide cannula. Ten to thirty minutes later, at a time when systemic cardiovascular function was stabilized and returned to the baseline levels, 0.1 μl of 0.9% NaCl vehicle or rat $\beta$-endorphin (Peninsula Laboratories, Inc., Belmont, Calif.) solution was administered into the PVH over 2 minutes using a microinjection pump (model CMA/100, Carnegie Medicin AB, Stockholm, Sweden)-driven 10-μl syringe (Hamilton Co., Reno, Nev.) attached to the injector by a short length of PE-10 tubing, with Teflon tubing comprising the remaining portion. Drug delivery was precisely controlled by measuring the length of movement of the oil/aqueous interface.
along a calibrated 5 μl glass pipette (Accu-Fill 90, Clay Adams) interposed in the Teflon tubing, using a micro-slide field finder (Teledyne Gurley, Troy, N.Y.) under the light microscope. Drug delivery was further confirmed by the movement of a small air bubble introduced into the PE-10 tubing. The injector was allowed to remain in place for a total of 5 minutes after the initiation of drug injection. Three additional blood samples were taken at 15, 30, and 60 minutes after PVH injection.

Two to five days after the conscious protocol, each rat was anesthetized with α-chloralose (60 mg/kg i.v.; Sigma Chemical Co., St. Louis, Mo.) after halothane induction. Body temperatures were stabilized (37°–38.5°C) by maintaining each animal on an activated Deltaphase Isothermal Pad (Braintree Scientific, Inc., Braintree, Mass.). Blood pressure and heart rate were measured for an hour after PVH injection of 0.9% NaCl or β-endorphin.

In naltrexone antagonism studies, after control blood sampling, 0.9% NaCl or naltrexone-HCl (1.1 μg/0.1 μl over 2 minutes; Sigma) was injected into the PVH about 20 minutes before injection of 0.9% NaCl or β-endorphin. An additional blood sample was taken 30 minutes after the final PVH injection.

On termination of an experiment, each rat was anesthetized with sodium pentobarbital (40 mg/kg i.p.) and perfused through the left cardiac ventricle with 0.9% NaCl (50 ml) followed by 10% phosphate-buffered formalin (50–100 ml). Frozen sections of each brain were cut (40 μm) on a cryostat (model D, Ames Lab-Tek, Inc., Westmont, Ill.), were stained with cresyl violet, and were examined under the light microscope to visualize placement of the tip of the injector. Estimates of the extent of diffusion of β-endorphin after PVH injection were made with a radioisotopic method. A working solution was prepared by mixing [125I]β-endorphin (3-4 [125I]iodotyrosyl-β-endorphin; specific activity 2,013 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) with rat β-endorphin to a final concentration of 3.5 μg/0.1 μl. Rats were decapitated 30 minutes after injection of 0.1 μl of the working solution into the PVH of pentobarbital-anesthetized rats over 2 minutes. After cutting serial 100-μm sections in either the sagittal, coronal, or horizontal planes on a cryostat, each section was transferred into a 5-ml polystyrene culture tube, and then the radioactivity was measured by a Beckman gamma 5500 counter (Beckman Instruments, Inc., Irvine, Calif.).

Plasma norepinephrine and epinephrine were separated by alumina extraction and measured by high-performance liquid chromatography with electrochemical detection (model 200, Bioanalytical Systems, Inc., West Lafayette, Ind.). Plasma glucose concentrations were measured using a commercially available hexokinase kit (Glucose-SR Liquid Stable Reagent Set, Medical Analysis Systems, Inc., Camarillo, Calif.).

Data from individual animals were included in experimental analyses only when the tip of the injector tract was located within or along the border of the PVH as defined by Armstrong et al. Overall statistical significance among various groups was tested using analysis of variance (ANOVA) techniques, including one-way or two-way ANOVA. When appropriate, the Duncan's multiple range test was used for specific comparisons. A p<0.05 was considered statistically significant. Data are expressed as mean±SEM.

Results

Effects of β-Endorphin on Behavior

Unilateral injections of β-endorphin (3.5, 1.0, and 0.1 μg) into the PVH of conscious rats produced excitatory behavior primarily consisting of hyperactivity, alerting, and occasional grooming. The excitatory behavior started several minutes after β-endorphin injections and continued for more than an hour in SHR, whereas it started 25–45 minutes after β-endorphin injections in WKY rats. Moreover, SHR appeared to be more excited than WKY rats. Simply after insertions of a stainless-steel injector filled with a β-endorphin solution of 3.5 μg/0.1 μl into the PVH, most SHR continued to show excitatory behavior for up to several hours, which made injections with this dose of β-endorphin difficult. Thus, the lower doses of β-endorphin were used in the following studies. Figure 1 shows a coronal section through the hypothalamus depicting the location of an injection site in the PVH. The lesion was made by the tip of a 33-gauge stainless steel injector.

Effects of β-Endorphin on Mean Blood Pressure and Heart Rate

In a resting state, SHR in all treatment groups had significantly greater baseline mean blood pressure (MBP) values as compared with WKY rats, whereas baseline heart rate values were comparable between strains (Table 1). Figure 2 illustrates time-course changes in MBP and heart rate after unilateral injections of 0.9% NaCl or β-endorphin (0.1 and 1 μg) into the PVH of conscious SHR and WKY rats. Injections of 0.9% NaCl vehicle did not alter either MBP or heart rate significantly in either strain. Injections of both doses of β-endorphin into the PVH produced significant increases in MBP and heart rate in both strains compared with vehicle-treated groups. However, at both doses of β-endorphin, the increases in MBP and heart rate were significantly greater in SHR compared with WKY rats. Moreover, MBP and heart rate increased rapidly and reached a plateau at about 15 minutes after injections of β-endorphin in SHR, whereas MBP and heart rate increased slowly and gradually until 45 minutes in WKY rats. Both doses of β-endorphin produced comparable increases in MBP in either SHR or WKY rats, whereas the plateau level of heart rate produced by a 0.1-μg dose of β-endorphin was about twice that produced by a 1-μg dose of β-endorphin in SHR.

Two to five days after the study in conscious rats, they were anesthetized with α-chloralose following
halothane induction. Under the anesthesia, there were substantial decreases in baseline MBP values in both SHR and WKY rats, whereas baseline heart rate values remained unchanged compared with those in conscious animals (Table 1). Baseline MBP values were still significantly greater in SHR than in WKY rats. Unilateral PVH injections of a 0.1-μg dose of β-endorphin tended to produce gradual, minor increases in MBP of both strains without affecting heart rate compared with each saline-treated group (Figure 3). The minor increases in MBP reached a statistical significance only in SHR. However, there were no significant differences in MBP or heart rate between SHR and WKY rats after β-endorphin injections.

Finally, ganglionic blockade with pentolinium completely blocked β-endorphin-induced pressor responses while partially blocking β-endorphin-induced tachycardiac responses in conscious SHR (Table 2). Moreover, during pentolinium treatment, PVH injections of β-endorphin produced only very minor behavioral excitation.

### Table 1. Baseline Cardiovascular and Plasma Catecholamine and Glucose Values in Conscious Spontaneously Hypertensive Rats and Wistar-Kyoto Rats and Baseline Cardiovascular Values in Anesthetized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strain</th>
<th>n</th>
<th>MBP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>NE (pg/ml)</th>
<th>EPI (pg/ml)</th>
<th>Glucose (mg%)</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Saline</td>
<td>WKY</td>
<td>8</td>
<td>103±2</td>
<td>348±7</td>
<td>188±28</td>
<td>151±26</td>
<td>150±5</td>
<td>80±5</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>7</td>
<td>138±4*</td>
<td>345±14</td>
<td>251±18</td>
<td>154±44</td>
<td>147±6</td>
<td>102±3*</td>
</tr>
<tr>
<td>β-E 0.1 μg</td>
<td>WKY</td>
<td>8</td>
<td>104±2</td>
<td>352±8</td>
<td>176±16</td>
<td>144±15</td>
<td>147±6</td>
<td>70±3</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>6</td>
<td>137±1*</td>
<td>361±6</td>
<td>176±30</td>
<td>175±17</td>
<td>150±6</td>
<td>95±3*</td>
</tr>
<tr>
<td>β-E 1 μg</td>
<td>WKY</td>
<td>6</td>
<td>103±5</td>
<td>350±14</td>
<td>149±22</td>
<td>106±22</td>
<td>134±7</td>
<td>...</td>
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<tr>
<td></td>
<td>SHR</td>
<td>5</td>
<td>132±4*</td>
<td>335±10</td>
<td>157±20</td>
<td>128±21</td>
<td>143±7</td>
<td>...</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. n, number of animals in each group; MBP, mean blood pressure; HR, heart rate; NE, norepinephrine; EPI, epinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; β-E, β-endorphin.

*p<0.05 SHR compared with WKY rats in each treatment group.
Effects of β-Endorphin on Plasma Concentrations of Catecholamines and Glucose

Baseline plasma concentrations of norepinephrine, epinephrine, and glucose were not significantly different between strains in all treatment groups (Table 1). Figure 4 illustrates the effects of vehicle or β-endorphin injected into the PVH on plasma norepinephrine and epinephrine concentrations at 15, 30, and 60 minutes after PVH injections. Injections of saline into the PVH produced no significant effects on plasma catecholamine levels in either strain. Injections of β-endorphin (0.1 and 1 μg) into the PVH produced minor, but not significant, increases in plasma norepinephrine concentrations compared with a saline-treated group in WKY rats, whereas plasma norepinephrine concentrations significantly

![Graph showing changes in blood pressure and heart rate over time](http://hyper.ahajournals.org/)

**Figure 2.** Line graphs show effects of paraventricular hypothalamus (PVH) injections of saline (0.1 μl) or β-endorphin (β-E) (0.1 and 1 μg/0.1 μl) on mean blood pressure (MBP) and heart rate (HR) in conscious spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Left panels show results obtained with 0.1 μg of β-E and right panels those obtained with 1 μg of β-E. Data are plotted as the changes in MBP and HR compared with baseline values immediately before PVH injections. Baseline values are given in Table 1. Data are presented as mean ± SEM. Number of animals in each group is given in parentheses. Treatments were given at time 0. *p<0.05 compared with the time–response curve of the β-E–treated WKY rats by analysis of variance. +p<0.05 compared with the time–response curve of each saline control group.
increased with both doses compared with a saline-treated group in SHR, showing significant differences between strains. However, plasma epinephrine concentrations significantly increased in both strains compared with each saline-treated group with significantly greater (more than twofold) increases in SHR compared with WKY rats. In general, increases in plasma catecholamine concentrations reached a plateau at either 15 or 30 minutes after injections of \( \beta \)-endorphin and were maintained during the 1-hour experimental period. Doses of 0.1 and 1 \( \mu \)g of \( \beta \)-endorphin produced comparable increases in plasma norepinephrine and epinephrine concentrations in either strain except that 0.1 \( \mu \)g, as compared with 1 \( \mu \)g, produced a greater increase in norepinephrine concentration at 15 minutes in SHR. A 0.01 \( \mu \)g dose of \( \beta \)-endorphin produced slight, but not significant, increases in plasma norepinephrine and epinephrine concentrations in SHR.

The responses of plasma glucose concentrations to \( \beta \)-endorphin were similar to those of plasma epinephrine concentrations to \( \beta \)-endorphin. Injections of \( \beta \)-endorphin (0.1 and 1 \( \mu \)g) into the PVH significantly increased plasma glucose concentrations in both strains, compared with each saline-treated group, with a significantly greater (more than twice) increase in SHR only at the dose of 0.1 \( \mu \)g compared with WKY rats (Figure 5). The increases in plasma glucose concentrations by \( \beta \)-endorphin appeared to be dose-related in WKY rats, whereas the 0.1 \( \mu \)g dose of \( \beta \)-endorphin produced greater increases in plasma glucose concentrations than did the 1 \( \mu \)g dose in SHR. In general, the increases in plasma glucose concentrations reached a plateau at 30 minutes and were maintained for at least an additional 30 minutes. A 0.01 \( \mu \)g dose of \( \beta \)-endorphin produced little effect on plasma glucose concentrations in SHR.

When the data were expressed as maximal increases in plasma norepinephrine, epinephrine, and glucose concentrations after \( \beta \)-endorphin injections, the results were similar to those previously described in the time-course studies.

**Naltrexone Antagonism of \( \beta \)-Endorphin–Stimulated Cardiovascular and Sympathoadrenal Function in Spontaneously Hypertensive Rats**

To examine whether the \( \beta \)-endorphin–induced stimulatory responses were mediated through an action on opioid receptors in the PVH, the nonselective opioid receptor antagonist naltrexone (1.1 \( \mu \)g) or saline was injected unilaterally into the PVH about 20 minutes before saline or \( \beta \)-endorphin (0.1 and 0.01 \( \mu \)g) injection into the same side of the PVH in conscious SHR. Table 3 shows baseline MBP and heart rate values and baseline plasma norepinephrine, epinephrine, and glucose concentrations during the resting state before PVH injections. There were no significant differences among groups in those baseline levels. Figure 6 illustrates time course effects of naltrexone pretreatment on \( \beta \)-endorphin (0.1 \( \mu \)g)–induced increases in MBP and heart rate. Injections of naltrexone alone into the PVH produced no significant effect on either MBP or heart rate. Pretreatment with naltrexone (1.1 \( \mu \)g) at 100 times the molar dose of \( \beta \)-endorphin (0.1 \( \mu \)g) significantly delayed the onset of pressor and tachycardiac responses to \( \beta \)-endorphin. However, MBP and heart rate began to increase 20 minutes after \( \beta \)-endorphin injection in naltrexone-pretreated SHR and reached the levels seen in vehicle-pretreated SHR at about 45 minutes. The increases in MBP and heart rate were coincident with behavioral excitation. Injections of a 0.01 \( \mu \)g dose of \( \beta \)-endorphin did not produce a significant increase in either MBP or heart rate in SHR (data not shown).

Figure 7 shows effects of naltrexone pretreatment on \( \beta \)-endorphin–induced increases in plasma norepinephrine, epinephrine, and glucose concentrations, when sampled 30 minutes after the last PVH injections in SHR. Injections of naltrexone alone into the PVH produced no significant effect on plasma con-
centrations of plasma norepinephrine, epinephrine, or glucose. Naltrexone pretreatment substantially blunted the increases in plasma epinephrine and glucose but not the increases in plasma norepinephrine concentrations produced by PVH injection of β-endorphin (0.1 μg). Injections of β-endorphin at a dose of 0.01 μg did not produce significant increases in plasma catecholamine or glucose levels.

**Diffusion Patterns of β-Endorphin**

The extent of diffusion of the iodine-125 label was examined 30 minutes after injections of a mixture of [125I]β-endorphin and β-endorphin at a dose of 3.5 μg into the PVH. β-Endorphin appeared to diffuse away from the injection site in a symmetrical fashion in the sagittal, coronal, and horizontal planes, with a wider dorsal distribution in the horizontal planes. Most of the recovered radioactivity was detectable within ±0.5 mm from the injection site in the sagittal (93%), coronal (91%), and horizontal (72%) planes. More than half of the injected amount of β-endorphin appeared to be located within a cubic volume, 700 μm on each side, with the injection site at the center of that volume.

**Discussion**

The present results demonstrate that intracerebral injections of β-endorphin into the PVH stimulate cardiovascular and sympathoadrenal function in conscious rats. Furthermore, the sympathoadrenal axis is significantly more responsive to PVH β-endorphin injections in the developing SHR than in age-matched WKY rats.

Unilateral administration of β-endorphin into the PVH produced excitatory behavior similar to that produced by excitatory amino acid injection8,9 into, or electrical stimulation9 of, the PVH. Administration of β-endorphin in a dose of 10 or 20 μg into the third cerebral ventricle was reported to produce similar excitatory behavior in cats.29 Moreover, administration of β-endorphin in low doses (0.01–0.3 μg) into the third cerebral ventricle produced similar excitatory behavior in rats.30 Thus, it is possible that the excitatory behavior was produced by diffusion of β-endorphin into the periventricular area or the third ventricle. Since ganglionic blockade with pentolinium abolished most of the behavioral excitation produced by PVH injections of β-endorphin, the excitation
appeared to be mediated indirectly by increased sympahtoadrenal outflow. However, since behavioral excitation itself is known to affect cardiovascular function and was usually coincident with increases in MBP and heart rate, the possibility that the excitatory behavior contributed to pressor and tachycardic responses cannot be excluded.

Unilateral administration of β-endorphin into the PVH increased MBP and heart rate in both SHR and WKY rats, effects that were evident for more than an hour in both strains. These responses are consistent with the prolonged duration noted for the analgesic effect of β-endorphin. The lengthy nature of β-endorphin-mediated responses may be due to its relative resistance to peptidases or high affinity to and extremely slow dissociation from binding sites. The increases in MBP and heart rate occurred much more rapidly and were significantly greater in SHR compared with WKY rats. Spontaneously hypertensive rats have been reported to show enhanced pressor responses to intracerebroventricular leucine-enkephalin, intracerebroventricular [d-Ala2-Met]-enkephalin, and DAGO or [d-Ala2,d-Leu2]-enkephalin microinjected into the medial preoptic nucleus of the hypothalamus. Moreover, the renal and mesenteric vasoconstrictions produced by intracerebroventricular DAGO were found to be significantly enhanced in SHR compared with WKY rats. It is of some interest that the regional hemodynamic alterations produced by intracerebroventricular DAGO are similar to those produced by electrical stimulation of the PVH. In light of the facts that microinjection of either DAGO or β-endorphin into the PVH produced pressor and tachycardiac responses and stimulated sympathoadrenal outflow, the PVH may be considered as one of the sites of action of enkephalins administered intracerebroventricularly. The magnitude of tachycardiac responses to the highest dose (1 μg) of β-endorphin tested in the present study was less than that produced by the 0.1 μg dose of β-endorphin, especially in SHR. Since the plasma catecholamine responses to both doses of β-endorphin were comparable, the finding of reduced tachycardic responsiveness might be due to

Table 3. Baseline Cardiovascular and Plasma Catecholamine and Glucose Values of Conscious Spontaneously Hypertensive Rats in Naltrexone Antagonism Studies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>MBP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>NE (pg/ml)</th>
<th>EPI (pg/ml)</th>
<th>Glucose (mg%)</th>
</tr>
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<tbody>
<tr>
<td>NAL+saline</td>
<td>6</td>
<td>130±3</td>
<td>313±15</td>
<td>166±41</td>
<td>166±29</td>
<td>141±7</td>
</tr>
<tr>
<td>NAL+β-E 0.1 μg</td>
<td>5</td>
<td>131±2</td>
<td>320±6</td>
<td>147±31</td>
<td>142±20</td>
<td>145±5</td>
</tr>
<tr>
<td>Saline+β-E 0.1 μg</td>
<td>6</td>
<td>129±2</td>
<td>327±14</td>
<td>170±42</td>
<td>125±41</td>
<td>143±3</td>
</tr>
<tr>
<td>NAL+β-E 0.01 μg</td>
<td>4</td>
<td>123±2</td>
<td>296±13</td>
<td>93±6</td>
<td>156±28</td>
<td>147±4</td>
</tr>
<tr>
<td>Saline+β-E 0.01 μg</td>
<td>4</td>
<td>129±4</td>
<td>298±5</td>
<td>110±14</td>
<td>184±14</td>
<td>139±3</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. n, number of animals in each group; MBP, mean blood pressure; HR, heart rate; NE, norepinephrine; EPI, epinephrine; β-E, β-endorphin; NAL, naltrexone (1.1 μg) was injected into the paraventricular hypothalamus 20 minutes before saline or β-endorphin administration.
When the animals were anesthetized with α-chloralose, there were substantial decreases in baseline MBP in both SHR and WKY rats. Moreover, microinjections of β-endorphin into the PVH produced very minor increases in MBP without affecting heart rate of either strain. Finally, there were no significant differences in the magnitude of the changes in MBP and heart rate between strains after β-endorphin injections. Thus, the anesthetic at a dose (60 mg/kg i.v.) used in the present study seems to depress cardiovascular function to some extent, emphasizing the importance of using conscious animals. A number of studies have demonstrated differences in cardiovascular responses to centrally administered opioid agonists between conscious and anesthetized animals.  

The pressor and tachycardiac effects produced by PVH β-endorphin injections appear to be mediated primarily by centrally mediated increases in sympathoadrenal outflow, since ganglionic blockade with pentolinium blocked the β-endorphin–induced stimulatory cardiovascular responses. Microinjections of β-endorphin into the PVH produced increases in plasma concentrations of epinephrine in WKY rats, whereas increases in plasma concentrations of both norepinephrine and epinephrine were produced in SHR. Moreover, the increases in plasma concentrations of catecholamines produced by β-endorphin were significantly greater in SHR than in WKY rats, indicating that the enhanced pressor and tachycardiac responses in SHR might be produced in response to a greater central activation of sympathoadrenal outflow. In addition, increased vascular sensitivity to catecholamines, which has been reported in SHR, may also contribute to the enhanced pressor responses to PVH β-endorphin in SHR. There appear to be three likely explanations for differential sympathoadrenal regulation by PVH β-endorphin between SHR and WKY rats. First, the differential sympathoadrenal responses to PVH β-endorphin might be produced by differential sensitivity to β-endorphin between strains. This differential sensitivity might result from a difference in the density of opioid receptors. For example, SHR in the developmental phase of hypertension have demonstrated differences in cardiovascular responses to centrally administered opioid agonists between conscious and anesthetized animals.

![Graph showing antagonism by naltrexone of mean blood pressure (MBP) and heart rate (HR) responses to paraventricular hypothalamus (PVH) injections of β-endorphin (β-E) (0.1 μg/0.1 μl) in conscious spontaneously hypertensive rats (SHR).](image-url)
dorphin might result from a differential distribution of opioid receptors (within the PVH) between strains. Thus, in WKY rats, opioid receptors may be distributed on parvocellular PVH neurons that primarily regulate adrenal medullary function. However, in SHR opioid receptors may be distributed on parvocellular PVH neurons that regulate the sympathetic nervous system and adrenal medullary function. Spinally projecting parvocellular neurons have been shown to be organized in a topographic fashion that correlates with the rostrocaudal distribution of the ganglion-specific sympathetic preganglionic neurons. Finally, norepinephrine and epinephrine secretions from the adrenal medulla might be differentially regulated by adrenal sympathetic stimulation between strains. Further studies will be necessary to elucidate the mechanism of the differential sympathoadrenal responses to PVH β-endorphin between strains.

The responses of plasma glucose concentration to β-endorphin were similar to those of plasma epinephrine concentration to β-endorphin, suggesting that the hyperglycemic effects of β-endorphin might be mediated, at least in part, by increased secretion of epinephrine from the adrenal medulla. Intracisternal administration of β-endorphin also produced hyperglycemia mediated by increased central sympathetic outflow to adrenal medulla. In addition, administration of β-endorphin into the third cerebral ventricle produced strong and long-lasting hyperglycemia. Moreover, intracerebroventricular administration of naloxone has been shown to suppress hyperglycemia induced by intracerebroventricular administration of 2-deoxy-D-glucose, a glucose analogue that competitively inhibits glucose use, suggesting that endogenous opioid peptides play a role in the central regulation of glucose homeostasis.

Administration of naltrexone (1.1 µg) alone into the PVH produced little effects on MBP, heart rate, and plasma catecholamine concentrations in SHR, suggesting that opioid systems in the PVH may not play an etiological role in the development of hypertension in SHR. Instead, this observation suggests that the β-endorphin system in the PVH plays an integrative rather than a tonic primary role in cardiovascular and sympathoadrenal regulation. The initial pressor and tachycardiac responses to and the adrenal medullary stimulation by PVH injection of β-endorphin (0.1 µg) were substantially blocked by pretreatment with naltrexone (1.1 µg) at 100 times the molar dose of β-endorphin injected into the PVH, suggesting that the stimulatory effects of β-endorphin were mediated primarily by an action on naltrexone-sensitive opioid receptors in the PVH. However, the delayed pressor and tachycardiac responses to and the increases in plasma norepinephrine concentrations by β-endorphin were not blocked by pretreatment with naltrexone injected into the PVH. Moreover, the delayed pressor and tachycardiac responses were coincident with behavioral excitation. Hyperactivity, resulting from morphine administered into the periaqueductal gray matter, was also blocked only temporarily (about 10 minutes) by naloxone given before or after the morphine injection. Thus, it appears that there is a nonspecific component to behavioral responses produced by opioids. Similarly, most of the inhibitory neuronal responses to mor-
phrine or Met-enkephalin iontophoretically applied into the hippocampus or thalamus were resistant to naloxone blockade, suggesting nonspecific actions of opioids. The reasons for the nonspecific actions of opioids are not clear. However, one possibility is that iontophoretic applications of drugs produce extremely high concentrations adjacent to receptor sites, causing nonspecific (i.e., naloxone-resistant) responses. Moreover, inhibition of spontaneous neuronal activity produced by morphine, [D-Ala²-Met]-enkephalin, or β-endorphin was antagonized by naloxone in some, but not all, PVH neurons tested from hypothalamic slices in vitro. Since β-endorphin was about 10 to 100 times more potent than morphine or the enkephalin analogue in reducing excitability, it was suggested that the responses that were resistant to naloxone blockade, represented interactions of opioids with a different type of opioid receptor rather than nonspecific opioid actions. In addition, naloxone injected into the PVH together with DAGO at 10 times the molar dose of DAGO, only partially antagonized the stimulatory effects of DAGO on plasma catecholamines measured 25–35 minutes after PVH injections. Moreover, β-endorphin has binding characteristics distinct from those of other opioid agonists, in that divalent cations inhibit β-endorphin binding in brain, suggesting a difference between β-endorphin and alkaloid/enkephalin binding sites. Thus, it is likely that there are naltrexone-resistant β-endorphin receptors in the PVH. Another possible explanation is that naltrexone, due to its clearance from the PVH, no longer maintains a concentration to block β-endorphin-induced stimulatory responses 20 minutes after β-endorphin injection. This possibility becomes less likely if the stimulatory responses to β-endorphin are mediated through an action on classical opioid receptors, since naltrexone has similar affinities for μ- and δ-opioid receptors compared with β-endorphin. Maximal antagonism by naloxone of the cardiovascular effects of β-endorphin was reported to occur 30–40 minutes after the antagonist was administered into the nucleus tractus solitarius. However, if the effects of β-endorphin are mediated through an action on a specific β-endorphin receptor (presumably the so-called e-receptor), the possibility still seems likely, since β-endorphin is about 63 times more potent than naloxone in displacing [³H]β-endorphin in rat brain membrane preparations. Naltrexone is only several times more potent for μ- and δ-opioid receptor blockades than naloxone, although relative potencies for a specific β-endorphin receptor between these two antagonists are not known. In addition, naltrexone is more lipophilic than β-endorphin, suggesting its more rapid removal into the cell than β-endorphin. However, the possibility remains to be resolved since higher doses of naltrexone were not tested in the present study. Another alternative possibility may be that β-endorphin concentration in the PVH was so high after injections of the 0.1 μg dose that it could displace naltrexone bound to opioid receptors. Approximate average β-endorphin concentration in areas surrounding the PVH at 30 minutes after β-endorphin (0.1 μg) injection appears to be several orders of magnitude greater than the IC₅₀ value (2.6 nM) to displace [³H]naloxone binding by β-endorphin in a rat brain membrane preparation. The approximate β-endorphin concentration was calculated based on the diffusion data. Thus, the delayed onset of β-endorphin-induced stimulatory responses after naltrexone pretreatment may reflect the time spent recruiting neurons, with receptors interacting with β-endorphin, sufficient to elicit the responses. However, this possibility seems unlikely since β-endorphin produced only minor, but not significant, effects when the dose of β-endorphin was decreased only by 10 times. Since feeding with highly palatable diets rich in sucrose has been shown to activate hypothalamic β-endorphin pools and sucrose feeding has been shown to stimulate central sympathetic outflow, we hypothesize that at the level of the PVH, β-endorphin integrates sympathoautoregulatory outflow and contributes to exacerbation or acceleration of the development of hypertension in association with feeding. However, it remains to be resolved whether the β-endorphin system in the PVH is, in fact, activated by sucrose feeding.

Enhanced cardiovascular and sympathoautoregulatory responsiveness of SHR to β-endorphin in the developmental phase of hypertension may reflect differences in opioid receptors in the PVH between WKY rats and SHR. The density of opioid binding sites in the brain of SHR in the developmental phase of hypertension is about twice that measured in WKY rats. Moreover, SHR in the developmental phase of hypertension have a higher density of μ-opioid receptors with similar apparent dissociation constant in the hypothalamus as compared with WKY rats. The paraventricular hypothalamus has been shown to be involved in hypertension of SHR. For example, it has been suggested that the PVH plays an etiological or a compensatory role in hypertension of SHR. Likewise, the precise role of the PVH β-endorphin system in hypertension of SHR remains to be elucidated.

In conclusion, the present results indicate that the putative neurotransmitter β-endorphin produces stimulation of systemic cardiovascular function and sympathoautoregulatory outflow at the level of the PVH in conscious rats, and anesthetics blunt the cardiovascular responses to β-endorphin administered into the PVH. In addition, the regulatory influence of β-endorphin is differentially expressed between SHR and WKY rats during the developmental phase of genetic hypertension, indicating that this system may modulate the development of hypertension in SHR.

References


**KEY WORDS** • hypothalamus • endorphins • opioid peptides • catecholamines • blood pressure • heart rate • glucose • spontaneously hypertensive rats
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C B Jin and R W Rockhold

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