Opiate Receptors and Cardiovascular Control in Conscious SHR and WKY Rats

Giora Feuerstein, M.D., Robert L. Zerbe, M.D., and Alan I. Faden, M.D., F.A.C.P.

SUMMARY This study examined the cardiovascular, respiratory, and sympathetic effects of selective \(\mu\) and \(\delta\) opioid agonists microinjected into the hypothalamic nucleus preopticus medialis (POM) of conscious SHR and WKY rats. The \(\mu\) receptor agonist D-Ala\(^2\)-MePhe\(^4\)-Gly\(^5\)-ol-enkephalin (DAGO) at a dose of 0.6 or 6.0 nanomoles (Nmol) increased the blood pressure and heart rate in WKY rats. In SHR rats, the lower dose of DAGO similarly had a pressor effect whereas the higher dose was depressor; heart rate was increased only by the 6.0 nmol dose in these animals. In both SHR and WKY rats, this opioid caused respiratory acidosis and elevation of plasma norepinephrine (NE) and epinephrine (E); plasma vasopressin was reduced by the higher dose of DAGO. All of these effects of the \(\mu\) agonist were reversed by the opiate receptor antagonist naloxone (0.5 mg/kg, i.a.). The \(\delta\) opiate-receptor agonist D-Ala\(^2\)-D-leu\(^5\)-enkephalin at a dose of 6.0 or 20.0 nmol increased blood pressure and heart rate in both SHR and WKY rats without affecting respiratory variables. Plasma NE and EPI were elevated at the peak of thepressor period.

These studies suggest that the anteroventral hypothalamic region may be an important site in central autonomic regulation by opioid peptides. The \(\mu\)-receptor agonist was more potent than the \(\delta\) agonist in eliciting cardiovascular and respiratory effects and associated sympathetic activation. SHRs differed from the normotensive strain by their opposite (depressor) response to the higher dose of DAGO, a finding that may indicate a potentially different role of endogenous opioids and \(\mu\) receptors in central cardiovascular control in the spontaneous hypertensive rat.

The presence of opioids and opiate receptors in specific brain nuclei known to regulate cardiovascular activity\(^{1-3}\) and the potent effects of opioid peptides on blood pressure (BP) and heart rate (HR) when administered into the lateral cerebral ventricle\(^{4,5}\) or the cisterna magna\(^{7}\) suggest that opioid peptides may be important endogenous substances in central cardiovascular control.

Opioid peptides are also known to produce differential central cardiovascular effects in SHR as compared with normotensive rats indicating a potential role for these substances in the genesis of hypertension. Rockhold et al.\(^6\) have demonstrated that SHRs respond with a greater incidence in BP after injection of a stable met-enkephalin analog into the lateral ventricle (ICV), while a similar phenomenon was shown with leu-enkephalin in stroke-prone SHR.\(^4\)

However, recent studies from our laboratory suggest that the cardiovascular responses elicited by ICV administration of opioids may reveal little about the functions of these substances in cardiovascular control or dyscontrol since opioids have variable or opposite cardiovascular effects when microinjected into discrete brain nuclei.\(^1\) Thus, morphine sulfate causes hypotension when microinjected into the hypothalamic periventricular nucleus but pressor responses following microinjection into the neighboring nucleus preopticus medialis (POM).\(^4\) Similarly, microinjections of the selective \(\mu\) opiate receptor agonist D-Ala\(^2\)-MePhe\(^4\)-Gly\(^5\)-ol-enkephalin (DAGO) into the nucleus of the solitary tract cause pressor responses\(^9\) whereas this same agonist produces hypotension after injection into the nucleus ambiguus (Hassen et al., unpublished data). From these data we have concluded that the central cardiovascular effects of opioids are site-specific and may not be accurately predicted from responses obtained by ICV injections (which reflect summation of various effects obtained from multiple sites).

Moreover, determination of the precise role(s) of opioids in central cardiovascular control is complicated by the fact that there exist multiple classes of opiate...
receptors in the brain. We have approached these problems by microinjecting highly selective ligands for the various opiate receptor types into discrete brain regions. We have recently shown that predominantly μ opiate receptors appear to mediate cardiovascular responses in discrete hypothalamic nuclei of anesthetized or conscious rats.

In the present study, we have investigated the possible roles of the μ and δ receptors in mediating central cardiovascular activity in normotensive and hypertensive states by microinjecting highly selective opioid agonists into the POM of SHR and WKY rats. This hypothalamic site was selected since we have shown this site to be sensitive to the cardiovascular effects of opioids.

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats, 270–300 g (Zivic Miller, Pennsylvania), were anesthetized with halothane (2% in oxygen) and placed in a stereotaxic device. A stainless steel guide cannula (G27) was inserted through the skull and fixed with glue (Eastman 910) according to the following coordinates, in reference to the Bregma (A7190, König and Klippel): 13 AP = −0.1, L = 0.6, V = 2.5 mm. A stainless steel plunger was inserted into the cannula and sealed by a drop of silicone gel. In addition, a polyethylene catheter (PE 50) was inserted into the femoral artery and threaded under the skin of the back and exited at the neck cuff. It was secured by a spring wire (attached by an adhesive collar) from the back of the skull and outside the cage. The tubing was filled with 0.9% NaCl solution containing heparin (250 μml). The rats were housed individually in plastic cages (21 x 27 x 16 cm, W x L x H) with food and water ad libitum, for 24–36 hours.

On the day of experiments (48 hours after guide cannula implantation), the arterial line was attached to a pressure transducer (Narco RP 1500), and recording of blood pressure (systolic, diastolic, mean) and heart rate was done by the NarcoTrace 80 computerized dyngograph. Blood samples (0.4 ml) were withdrawn from the arterial line for plasma catecholamine assay and measurements of blood pH, pCO₂, and pO₂, (0.4 ml, Corning Medical, pH/blood gas 165/2). Each blood sample was replaced by an equal volume of fresh rat blood. The guide cannula plunger was removed prior to the start of the experiments.

Experimental Protocol

A 60-minute control period was allowed for control recordings before intrahypothalamic injections of the drugs. DAGO (kindly provided by Doctor Maurer, Sandoz, Basel, Switzerland) or DADL (Peninsula, California) was injected in 0.9% NaCl solution (1 μl, sterile, pyrogen-free) through a 30 g needle to a depth of 8.0 mm below the surface of the skull. While the rats were in their home cages, 1 μl was injected over 30 seconds; efficient injection was ascertained by following the movement of an air bubble in the polyethylene tubing. Saline injections had no effect on any of the cardiovascular parameters, as previously shown. Cardiovascular responses to the opiates were recorded for 30 minutes and after naloxone (Endo Labs), 0.5 mg/kg injected intrabdominally, and recordings continued for an additional 10 minutes. Blood samples were withdrawn at the control period, 25–30 minutes after drug administration and 5–10 minutes after naloxone injection.

At the end of the experiment, rats were sacrificed by intraperitoneal injection of T61 (euthanasia solution; American Hoescht, Somerville, New Jersey). The brain was removed from the skull, frozen on dry ice, and cut in a cryostat into 50 μl slices. Thionine (0.1%) stained sections were examined microscopically for exact localization (A7020-A6300) of the injection site according to König and Klippel. 13 The area of injection and the tract of the injection cannula are shown in Figure 1. Each rat received only one injection.

To estimate the distance of diffusion of the enkephalin injected into the POM, /H-DADL (40 Ci/nmol, 1 μCi/ml, 1 μCi/rat, Amersham) was injected in 1 μl into the POM, and 30 minutes later the brain was removed, frozen, and cut (100 μ thick slices) in a cryostat. The brain slices were then dissolved in scintillation medium for tritium assay. By using this technique, we found that approximately 30 minutes after injection there were 1640 cpm/100 μl slice in the injection site (confirmed by microscopic examination). At 1 mm rostral there were 198 cpm/100 μl slice, and at 1.5 mm, 124 cpm/100 μl slice. These data show that a gradient of 1:10 in tritium concentration exists 1 mm rostral to the injection site and 1:13.2 at 1.5 mm.

Plasma Norepinephrine and Epinephrine Assay

Blood samples were centrifuged (5000 g, 4°C, 5 min) and plasma (200 μl) mixed with 0.5 M HClO₄ (200 μl) containing 31.8 mM EGTA. After centrifugation (5000 g, 4°C, 5 min) aliquots of 200 μl of the supernatant were frozen at −20°C until epinephrine (E) and norepinephrine (NE) were assayed by the radioenzymatic thin-layer chromatographic procedure described previously. Briefly, the procedure used was as follows: the protein-free aliquots were incubated with catechol-O-methyltransferase and tritiated Sadenosylm ethionine. After incubation, the reaction was stopped by the addition of borate buffer (pH 8.0) containing authentic metanephrine and normetanephrine. The amines were extracted into toluene: isoamyl alcohol (3:2) and then into 0.1 M acetic acid. The radioactive products were separated by thin-layer chromatography and the appropriate areas separately scraped into counting vials. After periodate oxidation of the O-methylated compounds to vanillin, phosphor-containing toluene was added and tritium assayed by liquid scintillation spectrometry.

Assay of Vasopressin

Heparinized plasma (250 μl) was precipitated with two volumes of cold acetone and the supernatant then extracted with five volumes of cold petroleum ether.
After removal of the petroleum ether phase by suction, the acetone water phase was completely dried in a vacuum centrifuge (Savant-Speed-Vac, Hicksville, New York). Just prior to assay, the extracted material was resuspended in assay buffer (0.1 M sodium phosphate buffer, 0.3% NaCl, 0.1% bovine serum albumin, pH 7.6). The recovery using this method is 65%; no correction was made for extraction losses. Vasopressin content was measured in the extracted plasma by a previously described radioimmunoassay17 that can reliably detect 0.2 to 40 pg per tube of standard vasopressin (Calbiochem, La Jolla, California).

Results are expressed as means ± SEM in text and figures. Analysis of variance with repeated measures design (BMDP2V)18 was used to evaluate differences in the cardiovascular responses between treatment groups. The Student t test (unpaired) was used to test differences in plasma catecholamines, vasopressin, blood gases, and pH.

**Results**

**Effects of DAGO on Cardiovascular and Sympathetic Responses in SHR and WKY Rats**

In the control period, the blood pressure (BP) of the SHRs used in this study was 191 ± 10/131 ± 5 mm Hg (systolic/diastolic); the BP of the WKY rats in the control period was 146 ± 8/88 ± 5 mm Hg. The systolic and diastolic BPs of SHR were significantly different from the systolic and diastolic BP of WKY (p < 0.0001). The heart rate in the control period of the conscious resting SHRs was 355 ± 13 bpm (n = 35) and was not significantly different from the control period heart rate of WKY rats: 347 ± 5 bpm (n = 37). No significant differences between control period levels of BP and HR were found among the various experimental groups of SHR or WKY rats. Plasma NE of SHR (n = 39) and WKY (n = 35) were 277 ± 23 and 167 ± 15 pg/ml (p < 0.05), respectively, and plasma levels of E in conscious resting SHR and WKY rats were 122 ± 13 and 72 ± 10 pg/ml (p < 0.05) respectively.

Injection of the low dose of DAGO (0.6 nmol) caused an increment in blood pressure in both the SHR and WKY rats; the increases in systolic and diastolic BP were larger in SHRs (F = 9.13, p < 0.001 for systolic BP; F = 7.25, p < 0.001 for diastolic BP, fig. 2). The increase in blood pressure 5 minutes after DAGO injection was 40.4 ± 5.5 mm Hg in SHR and 10.6 ± 6.4 mm Hg in WKY (p < 0.01). This dose also increased heart rate in the WKY rats up to 120 ± 16 bpm, which was significantly higher than the SHR group (F = 2.39, p < 0.0452, fig. 2 C). Blood pH, pCO₂, and pO₂ of SHR and WKY rats were not different at the control period (fig. 3). In both groups, 0.6 nmol DAGO caused a decrease in blood pH and pO₂, and increased pCO₂ equally. However, no differences could be observed between the groups (figs. 3 A–C). Plasma E and NE of the SHR and WKY rats are given in table 1. DAGO administration increased the circu-

**FIGURE 1.** Schematic frontal (A) and sagittal (B) sections of cannula tips and injection tract (C). Coordinates (above) are taken from the topographic atlas of König and Klippel (see ref 13), which should be consulted for undesignated areas. CA = anterior commissure; F = n. paraventricularis; HA = anterior hypothalamic n.; CO = optic chiasma; horizontal bar represents a distance of 1 mm. Dashed areas are sites of cannula placements. L580 denotes sagittal section made 580 μm lateral to midline, which is also close to the lateral coordinate of the injection site. C = authentic injection site into the POM; tip of cannula above the POM (as seen by microscopic examination) is denoted by arrow. The cannula tract is seen throughout the lateral septal area. The area of injection in this site fits A6860.
Figure 2. Effect of DAGO (0.6 nmol injected into the POM) on blood pressure and heart rate of SHR (○—○) and WKY (●—●) rats. △ SBP = change in systolic blood pressure; △ DBP = change in diastolic blood pressure; △ HR = change in heart rate. Abscissa denotes time (min) from the injection. Number of rats in each group are given in brackets. Asterisks denote significant difference by analysis of variance, with repeated measures design as indicated in the text.

Figure 3. Effect of DAGO (0.6 nmol injected into the POM) on blood pH, pO₂, and pCO₂ of SHR and WKY rats. Blank columns denote WKY (n = 9); dashed columns denote SHR (n = 10). Control period denotes levels before DAGO administration. NOX = naloxone 0.5 mg/kg, given intravenously 30 minutes after DAGO. Asterisks denote level of statistical significance from control period: *p < 0.001.

Table 1. Effect of DAGO on Plasma Catecholamines of SHR and WKY Rats

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (pg/ml)</th>
<th>Epinephrine (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Control period</td>
<td>30 minutes after DAGO</td>
</tr>
<tr>
<td>DAGO 0.6 nmol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (11)</td>
<td>281 ± 33</td>
<td>943 ± 130</td>
</tr>
<tr>
<td>WKY (9)</td>
<td>207 ± 34</td>
<td>666 ± 119</td>
</tr>
<tr>
<td>DAGO 6 nmol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (7)</td>
<td>335 ± 39</td>
<td>1369 ± 188</td>
</tr>
<tr>
<td>WKY (7)</td>
<td>156 ± 24</td>
<td>1072 ± 249</td>
</tr>
</tbody>
</table>

Asterisks denote level of statistical significance between groups as follows: *p < 0.05; †p < 0.01.
DAGO = D-Ala²-MePhe⁴-Gly⁵-ol-enkephalin; number in parentheses denotes the number of rats in each group.
lating levels of E and NE in both the SHR and WKY groups; plasma E increased to much higher levels in the SHRs.

Injection of the higher dose of DAGO (6.0 nmol) to SHR and WKY rats showed diverse responses in the two groups: in the WKY rats, BP increased up to +34 ± 6 mm Hg, but in the SHRs, both the systolic and diastolic BP decreased below control levels (figs. 4 A and B) and were significantly different from the WKY group (F = 8.03, p < 0.0001 for systolic BP; F = 10.68, p < 0.0001 for diastolic BP). Heart rate was significantly elevated in both groups, but no difference was found between the heart rate response of SHR and WKY rats. Naloxone administration, 30 minutes after DAGO, elicited opposite responses in SHR and WKY rats. Thus, 10 minutes after naloxone, the mean arterial BP of SHR increased by +15.1 ± 2.5 mm Hg (p < 0.001) while the mean BP of the WKY rats decreased by −29.3 ± 11.2 mm Hg (p < 0.05). In addition, naloxone further increased the heart rate of SHRs by +49 ± 8 bpm (p < 0.001) while the heart rate of WKY rats was decreased by −94 ± 36 bpm (p < 0.02). Blood pH, pCO2, and PO2 levels were markedly affected by the higher dose of DAGO. Respiratory acidosis was denoted by low pH (less than 7.3, fig. 5 A) and high pCO2 (over 45 mm Hg, fig. 5 B) while severe hypoxia was denoted by oxygen tension of less than 50 mm Hg (fig. 5 C). However, these changes were of the same magnitude in both SHR and WKY rats.

It is also noteworthy that the respiratory rate determined by counting chest movements was not depressed by the high dose of DAGO even though blood gases indicated severe respiratory acidosis. Thus, the respiration rate of resting SHR (n = 6) was 83 ± 1 breaths/min at the control period and 90 ± 4 breaths/min 30 minutes after 6 nmol DAGO; the respiration rate was 118 ± 7 breaths/min 5 minutes after naloxone administration (p < 0.01, compared to control period). This same phenomenon was also observed in WKY rats.

Plasma E and NE of both SHR and WKY rats markedly increased after DAGO injection (table 1). The levels of both NE and E were much higher than those

**FIGURE 4.** Effect of DAGO (6.0 nmol injected into the POM) on blood pressure and heart rate of SHR. (○–○, n = 9) and WKY (●–●, n = 9). Ordinates in A-C are the same as in figure 1. Asterisks denote significant statistical difference between the groups by analyses of variance, with repeated measures design as denoted in the text.

**FIGURE 5.** Effect of DAGO (6.0 nmol injected into the POM) on blood pH, pO2, and pCO2 of SHR and WKY rats. Blank columns denote WKY (n = 7); dashed columns denote SHR (n = 8). Period of blood samples are explained in figure 3. Asterisks denote level of statistical significance: *p < 0.001. No differences were found between the groups.
found after the lower dose of DAGO; plasma E was significantly higher in SHR than WKY rats 30 minutes after DAGO, and NE levels after naloxone administration also were still higher in the SHR (table 1).

Plasma vasopressin (table 2) at the control period was not significantly different between SHR and WKY rats. DAGO (6.0 nmol) suppressed plasma vasopressin in both the WKY and SHR. Naloxone administration increased the circulating level of vasopressin in both the SHR and WKY rats, but no significant differences between SHR and WKY rats were noted in the effect of DAGO on plasma vasopressin.

**Effect of D-Ala²-D-Leu⁵-Enkephalin (DADL) on Cardiovascular and Sympathetic Responses in SHR and WKY Rats**

Injection of the lower dose of the δ agonist, DADL (6.0 nmol), increased BP in both groups (fig. 6 A and B). Systolic BP of SHR increased by +32 ± 9 mmHg (p < 0.01), and of WKY by +16 ± 4 mm Hg (p < 0.01). The increments of the diastolic BP were less pronounced (fig. 5 B). Although some tendency to a larger pressor effect from DADL was seen in the SHR, no statistical significance was found between the groups throughout the complete experimental period.

DADL (6.0 nmol) also caused tachycardia in both the SHR and WKY rats, yet the magnitude of these changes was the same (fig. 6 C). DADL, unlike DAGO, had no effect on blood pH, pCO₂, and pO₂ (table 3). In addition, naloxone administration 30 minutes after DADL (6 nmol) reduced the systolic blood pressure of SHR by −12.8 ± 1.4 (p < 0.01) mmHg and that of WKY by −6.3 ± 1.1 mm Hg (p < 0.05). Heart rate of SHR and WKY rats decreased by −44 ± 27 and −43 ± 14 bpm, respectively.

Plasma NE and E concentrations (table 4) were elevated after DADL (6.0 nmol) in both SHR and WKY rats. However, the increments of the circulating levels were significantly higher in the SHR. In both groups, plasma NE and E returned toward control levels 5 minutes after naloxone administration.

Injection of the higher dose of DADL (20 nmol) also caused increments in BP (figs. 7 A and B) but somewhat smaller than the pressor effect of 6.0 nmol DADL. However, the increase in BP was more pronounced in SHR than WKY (F = 6.46, p < 0.001 for systolic BP; F = 6.12, p < 0.001 for diastolic BP). Heart rate was also significantly elevated in both groups: +140 ± 18 bpm in SHR and +86 ± 14 bpm in WKY, but no significant difference was found in group/period analysis (F = 0.42, p < 0.832).

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Control period</th>
<th>30 minutes after DAGO</th>
<th>5 minutes after naloxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAGO 6 nmol (10)</td>
<td>8.3±1.1</td>
<td>6.3±0.5*</td>
<td>24.3±6.2†</td>
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<tr>
<td>DADL 20 nmol (3)</td>
<td>8.7±2.6</td>
<td>6.0±4.8</td>
<td>33.7±3.9‡</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAGO 6 nmol (9)</td>
<td>8.1±1.4</td>
<td>6.4±2.2*</td>
<td>64.0±15.9‡</td>
</tr>
</tbody>
</table>

Asterisks denote level of statistical significance (Student’s t test, paired) as compared to control period as follows: *p < 0.05; †p < 0.02; ‡p < 0.01. Numbers in parentheses denote number of rats in each group.

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Control period</th>
<th>30 Minutes after DADL</th>
<th>5 Minutes after naloxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR (7)</td>
<td></td>
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<tr>
<td>pH</td>
<td>7.51±0.01</td>
<td>7.50±0.02</td>
<td>7.52±0.02</td>
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<tr>
<td>pO₂ (mm Hg)</td>
<td>79.7±1.3</td>
<td>77.8±2.2</td>
<td>88.1±2.1</td>
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<tr>
<td>pCO₂ (mm Hg)</td>
<td>31.6±1.1</td>
<td>31.3±1.4</td>
<td>30.3±1.1</td>
</tr>
<tr>
<td>WKY (7)</td>
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<tr>
<td>pH</td>
<td>7.49±0.01</td>
<td>7.51±0.02</td>
<td>7.51±0.01</td>
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<tr>
<td>pO₂ (mm Hg)</td>
<td>79.0±4.6</td>
<td>76.9±2.0</td>
<td>84.9±1.9</td>
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<tr>
<td>pCO₂ (mm Hg)</td>
<td>35.8±1.0</td>
<td>34.3±1.3</td>
<td>34.4±1.3</td>
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</table>

Numbers in parentheses denotes the number of rats in each group.
Table 4. Effect of DADL on Plasma Catecholamines of SHR and WKY Rats

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>30 minutes after DAGO</th>
<th>5 minutes after naloxone</th>
<th>Epinephrine (pg/ml)</th>
<th>30 minutes after DAGO</th>
<th>5 minutes after naloxone</th>
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<tbody>
<tr>
<td>DAGO 6 nmol</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SHR (7)</td>
<td>348 ± 49</td>
<td>802 ± 195*</td>
<td>439 ± 52</td>
<td>143 ± 58</td>
<td>549 ± 99*</td>
<td>186 ± 43</td>
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<tr>
<td>WKY (9)</td>
<td>206 ± 31</td>
<td>289 ± 55</td>
<td>223 ± 37</td>
<td>91 ± 46</td>
<td>196 ± 43</td>
<td>104 ± 39</td>
</tr>
<tr>
<td>DAGO 20 nmol</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>SHR (9)</td>
<td>143 ± 29</td>
<td>510 ± 80</td>
<td>288 ± 24</td>
<td>93 ± 19</td>
<td>691 ± 127</td>
<td>296 ± 52</td>
</tr>
<tr>
<td>WKY (9)</td>
<td>130 ± 24</td>
<td>334 ± 76</td>
<td>190 ± 41</td>
<td>71 ± 23</td>
<td>577 ± 195</td>
<td>246 ± 58</td>
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</table>

Asterisk denotes level of statistical significance between groups: *p < 0.02. DADL = D-Ala2-D-Leu5-Enkephalin.

Numbers in parentheses denote the number of rats in each group.

Blood pH, pO2, and pCO2 of SHRs were not affected by the higher dose of DADL (Table 5). In WKY rats, a significant decrease of blood pO2 (Table 5) was found 30 minutes after DADL administration and was reversed by naloxone.

The higher dose of DADL also caused large increments in plasma NE and E (Table 4) in the WKY and SHRs, but no significant difference was found between SHR and WKY rats.

Discussion

The present study demonstrates that the anteroven- tral third ventricle region of the rat hypothalamus (AV3V) is a sensitive site for the cardiovascular and respiratory effects of opioids. Both the μ and δ agonists produced cardiovascular changes via this region. In contrast, only DAGO caused respiratory effects. Since DAGO has very high selectivity for μ receptors in both bioassays and in binding assays, the ability of DAGO but not DADL to alter respiratory variables is consistent with the conclusion that μ rather than δ opiate receptors mediate the respiratory activity. Moreover, the profound respiratory effects of subnanomole doses of DAGO injected into the POM suggests the potential importance of this anteroven-tal hypothalamic region in mediating central respiratory effects of opioids.

The primary cardiovascular effects elicited by both the μ and δ agonists in SHR and WKY rats were to increase blood pressure and heart rate. These responses are consistent with the increment in blood pressure and heart rate following enkephalin injection into the cerebroventricular space. However, in the

Figure 7. Effect of DADL (20.0 nmol injected into the POM) on blood pressure and heart rate of SHR. (○—○, n = 9) and WKY (●—●, n = 9). Abscissa and ordinate are the same as in figure 1. Asterisks denote statistical significance between SHR and WKY by analysis of variance, with repeated measures design as given in the text.
previous studies severalfold greater doses of enkephalins (e.g., leu-enkephalin or D-Ala²-Met⁵-enkephalin) were used to elicit similar pressor responses by the ICV route of injection.⁴ ⁵ The more potent responses obtained by parenchymal microinjections therefore suggest that this hypothalamic region participates in mediation of the pressor effects observed after cerebroventricular injection of enkephalins.

In this regard, it should also be pointed out that it is unlikely that other opiate receptor types (e.g., κ or ε) mediate the effects of DAGO or DADL since, in vivo, the putative κ agonist Dynorphin 1–13, has a different profile of autonomic effects when microinjected into the same hypothalamic region,⁸ while β-endorphin, the putative ε receptor agonist, produces hypotension and bradycardia in conscious rats.²²

The maximal pressor response produced by 0.6 nmol DAGO was more pronounced than the pressor effects of the higher doses of DADL; this observation supports the view that μ receptors mediate the pressor effect of these enkephalins in this region. This finding is in contrast to the previous studies in which the pressor/tachycardic effects of ICV enkephalins were attributed to δ opiate receptors in the forebrain;¹¹ in those studies, the opioid peptides used were not as selective for specific opiate receptors. However, the present studies do not rule out the possibility that both μ and δ opiate receptors in the POM mediate similar cardiovascular effects with the quantitative differences resulting from differences in receptor number and/or affinity.

The highly selective μ agonist utilized in our experiments caused a depressor effect in SHRs at a dose of 6 nmol, whereas the lower dose (0.6 nmol) had a pressor effect; in contrast, significant pressor responses were seen in WKY animals at both doses. This may reflect activation of a separate depressor site or receptor group in SHRs. Consistent with this conclusion is the observation that in this experimental group naloxone injection significantly increased BP and heart rate. That DAGO may activate a cardiac decelerating mechanism may also be inferred by the lack of an increase in heart rate in SHRs treated with the low dose of DAGO, despite marked activation of the sympatheo-adrenomedullary system, as revealed by the high plasma levels of NE and E.

The cardiovascular differences produced by the two doses of DAGO may reflect subclasses of μ opiate receptors, which have recently been demonstrated by binding studies.²³ Thus, the cardiovascular differences between the SHR and WKY rats to exogenous opioids may suggest an altered sensitivity to the effects of endogenous opioid(s), which may reflect differences in μ receptor subtypes. Further studies are needed to characterize the types and number of opiate receptors and the endogenous opioid substances that are present in this hypothalamic region in these different rat strains.

The mechanisms involved in the pressor action of DAGO and DADL appear to be mediated by increased sympathetic tone. This finding is consistent with recent studies by Van Loon et al.²⁴ ²⁵ showing increased sympathetic activity following ICV administration of β-endorphin or D-Ala²-Met⁵-enkephalin in conscious rats. Furthermore, opioid peptides were shown to inhibit the release of NE from nerve endings, synapses, brain slices in vitro,²⁶ ²⁷ and brain nuclei in vivo.²⁸ In the brain, including the hypothalamus, opioid and adrenergic neurons are closely interrelated,²⁹ and colocalization in the same neural element was also suggested. Since injection of NE or other α-adrenergic agonists into the cerebroventricular system or directly into hypothalamic nuclei results in hypotension and bradycardia, it is possible that modulation (decrease) of NE release from adrenergic nerve ending by endogenous opioid peptides suppresses the inhibitory role of the hypothalamic adrenergic system on cardiovascular control and hence the pressor phenomenon. Our study provides further information as to the possible site of central sympathetic activation and the receptor subtypes involved in mediating these effects, since the magnitude of the increased circulating catecholamines after low doses of DAGO exceeded those found in the DADL-treated rats at much higher doses. Although the sympathetic response might be enhanced by the respiratory depression induced by DAGO, the respiratory acidosis is probably not the major factor in the increased sympathetic activity by the opioid peptides since no changes in blood pH or gases were found in DADL-treated rats, yet plasma catecholamines were significantly elevated. Furthermore, the greater increments in plasma NE and E of the SHRs after both DAGO or DADL indicates that the enhanced pressor response of SHR is not merely the result of the well-known supersensitivity of the peripheral resistance vessels to increased sympathetic activity,²² ³¹ but may be primarily due to the greater central activation of the sympathetic nervous system. Thus, our findings raise the possibility that the anteroventral POM region is a site of central sympathetic control by opioid peptides and is hyperreactive in the SHRs.

The present study also shows that vasopressin did not contribute to the pressor phenomenon of DAGO or DADL. Plasma vasopressin at the control period did not differ between SHR and WKY rats, which is in contrast to earlier data by Crofton et al.³³ However, plasma vasopressin decreased in the DAGO-treated SHR and WKY rats. These findings corroborate recent data by Rockhold et al.⁶ showing decreased plasma vasopressin after high dose of D-Ala²-Met⁵-enkephalin administered ICV. Interestingly, after naloxone administration, plasma vasopressin immediately rose above normal levels in both strains and especially in the SHRs. This rebound phenomena in the latter group might have contributed to the increase in blood pressure after naloxone injection to the DAGO-treated SHRs. Such a possibility, however, needs to be confirmed by a specific pressor antagonist of vasopressin.

In conclusion, the present study provides data that indicate specificity of both site and opiate receptor types in mediating the central cardiovascular and respiratory control by opioid peptides. This study also indicates that SHRs may be hyperreactive to central sym-
pathetic activation by opioid peptides; however, SHRs also display enhanced sensitivity to μ receptor-mediated depressor mechanism, which may reflect differences in μ receptor subtypes between SHR and WKY rats.

References

Opiate receptors and cardiovascular control in conscious SHR and WKY rats.
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