Enkephalin Effects on Blood Pressure, Heart Rate, and Baroreceptor Reflex

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SUMMARY The cardiovascular effects of opioid peptides have been studied. Leucine-enkephalin (Leu-ENK) produced blood pressure (BP) increases following administration into the lateral brain ventricles (i.v.t.), into the cisterna magna (i.c.m.), and following intravenous (i.v.) administration. Heart rate (HR) increases were observed following all routes of administration (threshold for BP and HR effects at 0.3 nmole, maximum at 360 nmol). The cardiovascular effects were independent of generalized seizures, which may occur at higher doses of enkephalins (ENK).

D-alanine-enkephalin (D-Ala-ENK) attenuated the vagal component of the baroreceptor reflex in cats. This was indicated by the findings that HR did not decrease following D-Ala-ENK-induced BP increases and that the compensatory decreases in HR following i.v. pressor doses of angiotensin II (ANG II) were markedly attenuated in cats treated with i.v.t. D-Ala-ENK.

Naloxone inhibited the BP and HR effects following i.c.m. and i.v., but not following i.v.t., administration of Leu-ENK. The i.v.t. Leu-ENK effects were inhibited by β-adrenergic receptor blockade. Brattleboro rats homozygous for hereditary diabetes insipidus with total absence of antidiuretic hormone (ADH) synthesis responded with BP decreases following i.v.t. Leu-ENK, while BP increases were observed in control Long-Evans rats.

Blood pressure increases to i.v.t Leu-ENK were markedly greater in spontaneously hypertensive rats of the stroke-prone strain (SHR-sp) than in normotensive control rats; SHR-sp exhibit a humoral pattern of increased ADH, ACTH, and catecholamines, presumably due to central peptidergic stimulation. The known effects of opioid peptides on these hormones and the observed cardiovascular responses suggest a possible participation of this peptide system in the maintenance of high BP in the SHR-sp.

(Hypertension 2: 395-407, 1980)

KEY WORDS • blood pressure • brain • enkephalins • naloxone • beta-blockade • spontaneously hypertensive rats

P EPTIDES such as endorphins, enkephalins, P and neurotensin are known to be widely distributed in peripheral tissue and in the brain. Several peptides previously believed to occur in peripheral tissue exclusively, however, namely vasoactive intestinal peptide, gastrin, kinin, and angiotensin, have only recently been discovered in the central nervous system. Angiotensin and kinins have been investigated for their central and peripheral effects on arterial BP, but little work has been done on the cardiovascular effects of other brain peptides.

Several observations suggest that opioid peptides may have an influence on the autonomic nervous system and on pituitary hormone secretion and, thereby, on BP regulation. First, it is well known that pain and stressful stimuli that entail important acute and chronic hemodynamic adaptation also activate the opioid peptide system. Second, opioid peptides were found in brain regions, e.g., the medial external layer of the median eminence, which are involved in the control of the secretion of hypothalamic and pituitary hormones. Enkephalins are localized in nerve fibers projecting from the hypothalamus to the posterior pituitary, where they could control the secretion of antidiuretic hormone (ADH). Third, the existence of a common precursor molecule of opioid peptides with adrenocorticotrophic hormone (ACTH), and the equimolar secretion of β-endorphin with ACTH argue in favor of a role of opioid peptides in vascular reactivity and in BP control. Fourth, the localization of enkephalin-like immunoreactivity in the nucleus tractus solitarii, a medullary area involved in cardiovascular regulation, suggests a role of the peptide in hemodynamic adaptation. Fifth, an interaction of opioid peptides with the sympathetic nervous system has been suggested, and brain adrenaline and noradrenaline are considered the most important neurotransmitters for BP control. Finally, changes in BP and heart rate

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following administration of morphine, opioid peptides, and their receptor antagonists have been demonstrated.\textsuperscript{4,9} \textsuperscript{14-17} We have therefore studied the central and peripheral cardiovascular effects of ENK in cats and rats. Spontaneously hypertensive rats have an increased responsiveness to another brain peptide, namely angiotensin II (ANG II);\textsuperscript{3} they have therefore been included in this study as well as rats with hereditary hypothalamic diabetes insipidus, which have an incapacity to synthesize ADH, thus permitting investigation of the contribution of ADH to ENK effects on BP.\textsuperscript{21} In addition, electrophysiological experiments were performed in cats, and pharmacological inhibition of ENK effects by naloxone and beta-adrenergic blockers were examined.

### Material and Methods

#### Experiments in Cats

Male cats weighing 2.5 to 3.5 kg were operated on under pentobarbital (Nembutal) anesthesia (40 mg/kg). Groups of four or more animals had stainless steel, glass-insulated electrodes implanted stereotaxically into limbic areas at an interpolar distance of 1 to 1.5 mm using the Horsley-Clarke stereotaxic apparatus. The following coordinates from the atlas of Reinoso-Suarez\textsuperscript{22} were used: from the central amygdaloid nucleus, frontal planes 12-14, lateral planes 7-9.5, and vertical level 6.5-7.5; from the hypothalamus, frontal planes 12, lateral planes 1-3, vertical level 6-7. The electrocorticogram (EEG) was taken from supra-sylvanian cortex.

Positions of the cannulae in the lateral brain ventricles (i.v.t.) (frontal plane 13, lateral plane 3) were checked by an ANG II drinking test.\textsuperscript{1} The electrode positions were verified at the end of the experiments by histological examination. At least 4 to 6 days were allowed for the animals to recover before the i.v.t. injections of peptides. Then D-Ala-ENK in doses of 170, 340, 3400, 34000, and 340000 nmoles per cat, were injected i.v. After 24 hours, the administration at the end of the experiments.\textsuperscript{14} The same type of cannula was placed in the cisterna magna through the occipital bone 2 mm above the membrana atlanto-occipitalis at an angle of 10°. The methods of brain cannulation and BP recording have been described in detail elsewhere.\textsuperscript{18} The rats were allowed a minimum of 6 days to recover from the operation. The day before testing, catheters were implanted under ether anesthesia into the femoral artery and femoral vein and tunneled under the skin to exit through the scarf of the neck. For testing, the animals were awake and freely moving in a wooden box (20 x 12 x 11 cm). Arterial BP was recorded via a Statham P23Db transducer and a Brush blood pressure computer on a Brush 2400 polygraph. Changes in BP were evaluated, and HR was calculated from the pulse pressure wave by the computer. Return of baseline cardiovascular parameters was attained before new drug injections. Under these conditions, no tachyphylaxis occurred.

Intravenous injections were given as a bolus of 200 \mu l, and the i.v.t. infusions lasted 10 minutes, with an infusion rate of 2 \mu l/min. For intracisternal injections, a maximal volume of 10 \mu l was used. Administration of the next dose was only started after BP and HR had returned to baseline levels.

In all experiments with propranolol or naloxone, the rat groups were divided: one half received the drug and the other half received control saline injections. This procedure avoided problems with possible tolerance following repeated Leu-ENK administration.

#### Intravenous Injections

If not stated otherwise, the peptides were injected in doses of 0 (0.9% saline control), 0.36, 3.6, 36, and 360 nmoles in a cumulative manner. The next dose was administered after return of BP and HR to baseline levels.

Normotensive Wistar-Kyoto (WKY) rats (n = 19) and spontaneously hypertensive rats (SHR-sp) (n = 18) were treated with Leu-ENK.

The effects of centrally and peripherally applied naloxone on intravenously administered Leu-ENK were tested. The SHR-sp (n = 10) and their normotensive WKY controls (n = 12) received a single injection of 36 nmoles Leu-ENK. Then, naloxone or saline was given i.v. by the same route, and 15 minutes later, the i.v. injection of 36 nmoles Leu-ENK was repeated.

Another group of normotensive WKY rats (n = 10) and SHR-sp (n = 10) were pretreated centrally with naloxone or saline i.c.i., and 30 minutes later, Leu-ENK was injected i.v.

A dose-response curve to i.v. Leu-ENK was established in normotensive WKY rats (n = 14); 30 minutes later, 1-propranolol (n = 7) or acidified saline (n = 7) was given i.v. After 24 hours, the administr-
tion of Leu-ENK was repeated in the propranolol-treated group and in the saline-treated control rats.

Naloxone alone was given i.v. to normotensive WKY rats (n = 8) and to SHR-sp (n = 9).

**Intraventricular Drug Administration**

Normotensive WKY rats (n = 13) and SHR-sp (n = 7) received i.v.t. Leu-ENK.

Both rat strains (WKY, n = 14; SHR-sp, n = 18) were treated i.v.t. with naloxone or saline and, 30 minutes later, with 36 nmoles of Leu-ENK.

Infusions of Leu-ENK were performed i.v.t. in SHR-sp (n = 22) and in WKY controls (n = 14); 30 minutes later, 1-propranolol or acidified saline was given i.v.t. to all rats. After 24 hours, at which time central beta-adrenergic blockade reportedly has its maximum, the administration of opioid peptides was repeated.

Naloxone was injected i.v.t. into normotensive WKY rats (n = 9) and into SHR-sp (n = 9).

**Intracisternal Injections**

Normotensive WKY rats (n = 11) and SHR-sp (n = 11) were treated i.c.i. with Leu-ENK.

Thirty minutes after i.c.i. pretreatment with naloxone or saline, Leu-ENK was injected by the same route into normotensive WKY rats (n = 10) and SHR-sp (n = 10).

**Rats with Hereditary Diabetes Insipidus**

Rats with hereditary hypothalamic diabetes insipidus from the Brattleboro strain (n = 6), which have a total incapacity for ADH synthesis, and Long-Evans control rats (n = 10) were challenged with 36 nmoles of Leu-ENK i.v.t.

**Drugs**

The drug naloxone hydrochloride was obtained from Endo Laboratories, Inc., New York, Lot. No. 78-050. It was dissolved in saline and injected at one single dose of 380 nmoles, if not indicated otherwise. This dose has been shown to be an effective blocking dose for cardiovascular and analgesic effects.** The drug 1-propranolol was obtained from ICI-Pharma, Plankstadt, Federal Republic of Germany, and always administered at one single dose of 386 nmoles. The Leu-ENK, Lot No. D 0843, and D-Ala-ENK, Lot No. D 0320, were purchased from Beckman Inc., Palo Alto, California. All peptides were dissolved in 0.9% saline, which was also used for the control infusions and injections.

**Statistical Analysis**

Results are reported as means ± standard error of the means. Student's t test and analysis of variances were used to evaluate statistical differences between the groups. Statistical significance was accepted at the 5% level.

**Results**

**Experiments in Cats**

Within a few minutes (3–8 min) after i.v.t. application of D-Ala-ENK at a dose of 425 nmoles, arterial systolic and diastolic BP increased by 28.7% ± 5.6% and 26.9% ± 4.6% respectively. Changes in HR were 7.4% ± 7.3%. The maximal hemodynamic response was observed 16 minutes after injection of the compound. The effect was attenuated after 64 minutes and was even reversed after 128 minutes (fig. 1). A dose of 170 nmoles D-Ala-ENK given i.v.t. had no effect on arterial BP and HR. In testing different commercial batches of D-Ala-ENK, up to 5 times higher potencies were noted. A dose of 850 nmoles increased arterial pressure by 52.5 ± 9.5 mm Hg. The sensitivity of the vagal component of the baroreceptor reflex was significantly decreased in cats that had received 425 nmoles D-Ala-ENK 60 minutes before testing of the baroreceptor reflex (fig. 2). It has been shown earlier in control experiments that a similar degree of HR variability as seen after the application of D-Ala-ENK has no marked influences on the testing of the vagal component of the baroreceptor reflex sensitivity.

The animals, especially at the highest dose of D-Ala-ENK (850 nmoles), displayed a catatonia-like behavior during the first 30 minutes. This was replaced by an excitatory behavior that lasted up to 2 hours. In the EEG recordings, there were dose-dependent hypersynchronous waves mainly within the amygdala and hippocampus. When the cats had received the highest dose of D-Ala-ENK, spike-wave complexes could be recorded within the amygdala and hippocampus (fig. 3). The time course of these EEG changes was different from that of the hemodynamic changes. The spike-wave complexes in the amygdala appeared approximately 5 minutes later than the hemodynamic changes and lasted for only 40 minutes.

**Experiments in Rats**

Control values of resting MAP were: in WKY, 106 ± 2.5 mm Hg; in SHR-sp, 160 ± 4.7 mm Hg; in rats with diabetes insipidus, 100 ± 3 mm Hg; and in Long-Evans rats, 83.8 ± 2.8 mm Hg. Resting HR was: in WKY, 316 ± 6 beats/min; in SHR-sp, 334 ± 9 beats/min, in rats with diabetes insipidus, 322 ± 8 beats/min, and in Long-Evans rats, 267 ± 9 beats/min. These values were not statistically different from resting BP and HR in experimental groups.

**Intravenous Injections in Rats**

The Leu-ENK caused dose-dependent rises in BP in normotensive WKY rats and in SHR-sp. These elevations were characterized by sharp onset and fast return to baseline levels (< 100 sec) (fig. 4). Significantly higher responses of BP and HR were observed in SHR-sp as compared to WKY rats (table 1).

Intravenous pretreatment with naloxone lowered BP and HR effects of 36 nmoles Leu-ENK significantly in WKY rats (fig. 5). In SHR-sp, naloxone equally reduced the Leu-ENK effects on BP from
45.2 ± 5.3 to 24.0 ± 3.3 mm Hg (p < 0.01) and on HR from +13.0 ± 5.1 to −3.0 ± 7.0 beats/min (p < 0.01). Higher doses of naloxone had no additional effects.

To investigate whether the peripheral effects of Leu-ENK were independent of central sites of action, naloxone pretreatment via the fourth brain ventricle was performed. In both WKY rats and SHR-sp, no differences in naloxone-treated animals as compared to the controls were observed. If propranolol was infused into the lateral brain ventricles of WKY rats, there was also no effect on the i.v.-induced cardiovascular responses of Leu-ENK. Naloxone given alone i.v. at doses of 0, 7.6, 76, 380, and 760 nmoles had no marked effects on BP and HR in WKY rats and in SHR-sp.

Intraventricular Infusions in Rats

Infusions of Leu-ENK into the lateral brain ventricles of WKY rats increased BP in a dose-dependent manner (table 2). These increases were different from the i.v. effects and were characterized by a slow onset and a long duration (fig. 4). Similar to the i.v. responses, SHR-sp showed a supersensitivity to i.v.t. Leu-ENK as compared to normotensive WKY rats (fig. 6). This difference was so marked that the highest dose Leu-ENK could not be continued in SHR-sp because of cerebral hemorrhage and behavioral changes. No significant differences in HR responses were observed between the two rat strains.

Naloxone did not antagonize the i.v.t. effects of Leu-ENK in WKY rats. The BP rose by 33.6 ± 5.6 mm Hg vs 37.1 ± 8.7 mm Hg before naloxone treatment. The respective HR increases were 35.0 ± 10.0 and 41.4 ± 29.4 beats/min. Similar results were obtained in the hypertensive rats.

For i.v.t. naloxone, the same doses were used as for the i.v. injections. Again, no particular effects on BP and HR were noticed in both rat strains. In contrast to naloxone, i.v.t. treatment with 386 nmoles of propranolol reduced BP rises following Leu-ENK infusions at the highest doses in SHR-sp and in WKY (fig. 7). The Leu-ENK-induced HR responses were significantly reduced by propranolol in SHR-sp, but not in WKY rats (fig. 7).

**FIGURE 1.** Changes in BP and HR (given as percent change of the corresponding control values) elicited by i.v.t. injection of D-Ala-ENK, 425 nmoles, in freely moving cats. Data are expressed as means ± SEM and have been obtained at different time intervals following the injections of D-Ala-ENK. Nine experiments were performed in four cats. In control experiments, using i.v.t. injections of the same volumes of 0.9% NaCl, changes in arterial BP did not exceed 5-10 mm Hg. Pretreatment values of BP and HR were 92 ± 3 mm Hg and 145 ± 3 beats/min respectively.
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FIGURE 2. Sensitivity of the vagal component of the baroreceptor reflex. Changes in the duration of pulse intervals (ordinate) are plotted against increases in SBP which were elicited by i.v. bolus injections of 0.1 μg angiotensin II, 60 minutes after i.v.t. application of 425 nmoles D-Ala-ENK. There was a significant change of the slope of the curve although the time course and degree of the pressure increases due to angiotensin II injections were not significantly altered. This indicates a decrease in baroreceptor sensitivity. Points on the curves represent means ± SEM of 16 experiments under control condition and of 17 experiments after administration of D-Ala-ENK. Control and experimental data were obtained in the same animals. Pretreatment values of HR in these experiments were 145 ± 3 beats/min, which corresponds to a pulse interval of 414 msec. At 60 minutes after injection of D-Ala-ENK and before injection of ANG II, HR was 133 ± 5 beats/min (451 msec pulse interval).

TABLE 1. Changes in Systolic Arterial Blood Pressure and Heart Rate Following Intravenous Leucine-Enkephalin (Leu-ENK) Injections

<table>
<thead>
<tr>
<th>Rats</th>
<th>BP, HR</th>
<th>Leu-ENK (nmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.36 3.6 36 360</td>
</tr>
<tr>
<td>WKY</td>
<td>BP</td>
<td>0.4 ± 0.5 0.3 ± 1.0 2.7 ± 0.6 20.2 ± 2.0 35.8 ± 2.7</td>
</tr>
<tr>
<td>(n = 19)</td>
<td>HR</td>
<td>3.3 ± 1.3 7.5 ± 2.3 10.6 ± 2.7 20.6 ± 4.7 24.7 ± 6.7</td>
</tr>
<tr>
<td>SHR-sp</td>
<td>BP</td>
<td>1.0 ± 0.5 1.7 ± 1.0 7.4 ± 2.5*§ 37.8 ± 3.2*§ 59.9 ± 4.0*§</td>
</tr>
<tr>
<td>(n = 18)</td>
<td>HR</td>
<td>3.8 ± 2.4 5.6 ± 3.4 11.4 ± 7.9 31.3 ± 6.2 53.0 ± 7.4*§</td>
</tr>
</tbody>
</table>

*p < 0.05.
†p < 0.01.
§Compared to WKY.
BP = blood pressure changes in mm Hg; HR = heart rate changes in beats/min.

TABLE 2. Changes in Systolic Arterial Blood Pressure and Heart Rate Following Intraventricular Leucine-Enkephalin (Leu-ENK) Administration

<table>
<thead>
<tr>
<th>Rats</th>
<th>BP, HR</th>
<th>Leu-ENK (nmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.36 3.6 36 360</td>
</tr>
<tr>
<td>WKY</td>
<td>BP</td>
<td>1.6 ± 1.2 1.7 ± 1.0 4.9 ± 2.0 14.9 ± 4.3 35.8 ± 6.3</td>
</tr>
<tr>
<td>(n = 13)</td>
<td>HR</td>
<td>6.6 ± 7.4 2.9 ± 9.0 21.2 ± 8.9 59.2 ± 19.8 58.1 ± 24.9</td>
</tr>
<tr>
<td>SHR-sp</td>
<td>BP</td>
<td>3.0 ± 2.3 3.0 ± 1.8 13.2 ± 7.6 49.7 ± 14.8†</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>HR</td>
<td>2.5 ± 1.6 7.5 ± 3.7 14.4 ± 6.4 55.0 ± 17.3</td>
</tr>
</tbody>
</table>

*p < 0.01.
†Compared to WKY.
BP = blood pressure changes in mm Hg; HR = heart rate changes in beats/min.
Figure 3. Recordings of the electroencephalogram (EEG) of central amygdala (Am. cent.), hypothalamus (Hypothal.), hippocampus (Hipp.), and lateral suprasylvian gyrus (sups. gyr. lat.), of heart rate (HR) (instantaneously recorded as intervals between two heart beats) and of arterial blood pressure (BP) before, immediately after, and 5, 10, 30, and 60 minutes after i.v.t. injection of D-Ala-ENK 850 nmoles, in a freely moving cat. The paper speed can be seen by the continuous marks on the top of each panel: each point represents 1 second. Five minutes (5') after application of the peptide, there is an increase in arterial pressure by approximately 20 mm Hg. At 10', arterial pressure is markedly increased by 30 mm Hg, and in the subcortical EEG recordings, there are hypersynchronous waves and spike-wave complexes. At 60 minutes after i.v.t. application of D-Ala-ENK, EEG recordings are not different from the control period; however, arterial BP is still elevated by 35 mm Hg.
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**Figure 4.** Direct recording of arterial BP (mm Hg) and of HR (beats/min) in rats following bolus injections of increasing doses of Leu-ENK intravenously (i.v.) (upper panel) and of 10-minute infusions into the lateral brain ventricles (i.v.t.) (lower panel). The i.v.t. cumulative doses were ineffective if infused over 10 minutes intravenously. This excludes the possibility of interference of i.v.t. Leu-ENK in the periphery. Chart speed was the same for recording of i.v. and i.v.t. responses.

**Figure 5.** Blood pressure and heart rate responses following i.v. injections of Leu-ENK (white columns) and of Leu-ENK combined with Naloxone (hatched columns) in normotensive WKY rats.
Following i.v.t. infusions of Leu-ENK, the rats behavior was altered; particularly at the highest dose of 360 nmoles, “wet-dog shakes,” which also resulted in BP spikes, could be observed (fig. 4). These were largely reduced by propranolol treatment.

**Intracisternal Injections in Rats**

Injections of Leu-ENK into the cisterna magna of normotensive WKY rats produced rises in BP and HR similar to those observed following i.v.t. treatment (table 3). In contrast to i.v. and i.v.t. infusions, Leu-ENK did not produce higher BP responses in SHR-sp than in WKY rats following i.c.i. administration (fig. 6). The HR increases in SHR-sp were also not higher than those in WKY rats. At the dose of 3.6 nmoles Leu-ENK i.c.i., BP and HR responses were even higher in WKY rats as compared to SHR-sp (table 3).

The 380 nmoles of naloxone, which did not produce any blocking effects when given i.v.t., lowered i.c.i. Leu-ENK-induced rises in BP and HR in WKY rats. At the dose of 36 nmoles, naloxone pretreatment reduced BP rises from 15.0 ± 1.7 to 8.5 ± 1.7 mm Hg (p < 0.05) and HR elevations from 28.0 ± 6.6 to 11.0 ± 5.8 beats/min (p < 0.05). Also, the effects of 360 nmoles of Leu-ENK, which produced increases in BP of 42.8 ± 6 mm Hg and in HR of 59.0 ± 11.4 beats/min, were significantly lower; after naloxone treatment, only rises of 17.2 ± 4.8 mm Hg (p < 0.01) and 20.0 ± 8.5 beats/min (p < 0.01) were noticed.

**Table 3. Changes in Systolic Arterial Blood Pressure and Heart Rate Following Intracisternal Leucine-Enkephalin (Leu-ENK) Injections**

<table>
<thead>
<tr>
<th>Leu-ENK (n mole)</th>
<th>WKY (n = 11)</th>
<th>SHR-sp (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.36</td>
</tr>
<tr>
<td>BP, HR</td>
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<td>WKY</td>
<td>BP</td>
<td>1.2 ± 0.6</td>
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<tr>
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<td>HR</td>
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<tr>
<td>SHR-sp</td>
<td>BP</td>
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</tr>
<tr>
<td></td>
<td>HR</td>
<td>2.7 ± 2.6</td>
</tr>
</tbody>
</table>

*p < 0.05.
†Compared to SHR-sp.

BP = blood pressure changes in mm Hg; HR = heart rate changes in beats/min.
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Figure 7. Blood pressure (upper panel) and heart rate responses (lower panel) following infusions of Leu-ENK into the brain ventricles of normotensive WKY rats (left) and SHR-sp (right). White columns represent cardiovascular parameters before propranolol treatment. Hatched bars represent values obtained with Leu-ENK 24 hours after i.v.t. pretreatment with I-propranolol.
The BP and HR responses following i.c.i. injections of Leu-ENK were equally reduced in SHR-sp by naloxone (fig. 8). In contrast to the i.v.t. treatment, only a few "wet-dog shakes" were observed following i.c.i. injections of Leu-ENK.

Rats with Diabetes Insipidus

The BP increase in Long-Evans rats following 36 nmoles of Leu-ENK i.v.t. was 10.7 ± 2.1 mm Hg, similar to that observed in WKY rats. In contrast, rats with diabetes insipidus, when given the same i.v.t. dose of Leu-ENK, did not respond with BP increases, but with significant BP decreases of -6.5 ± 2.0 mm Hg (table 4).

Discussion

Our results show that D-Ala-ENK in awake cats induces a cardiovascular response characterized by an increase in systolic and diastolic BP. The lack of a concomitant HR decrease when BP increased indicated that the vagal component of the baroreceptor reflex was attenuated. The degree of this attenuation was tested using i.v. injections of pressor doses of ANG II. It was observed that D-Ala-ENK reduced the sensitivity of the vagal component of the baroreceptor reflex to a similar degree as previously reported during electrical stimulation of amygdaloid and hypothalamic nuclei. The functional significance of this complex hemodynamic pattern elicited by i.v.t. administration of D-Ala-ENK is not clear. There are, however, striking similarities with the hemodynamic changes elicited by stimulation of limbic structures such as the amygdala. This gives rise to the possibility that enkephalins, which are present in the amygdala, are involved in the integration of psychomotor behavior and the concurrent hemodynamic adjustments. Enkephalins can induce epileptiform EEG activity when given i.v.t. The question of whether this could possibly cause some of the behavioral and cardiovascular effects can be answered by the fact that the EEG changes observed after i.v.t. D-Ala-ENK had a different time course and a different dose-effect relationship compared to the behavioral and hemodynamic changes. The BP changes were clearly independent of epileptic-like seizures.

Dose-dependent increases of BP and HR were also observed following central and peripheral administration of Leu-ENK in rats. Qualitatively similar results were obtained with D-Ala-ENK and with Met-ENK. The doses of D-Ala-ENK and of Leu-ENK used in these experiments are rather large, especially in cats. In rats, however, it appears that the BP bioassay of ENK is more sensitive than the one for analgesia. Analgesic threshold doses for i.v.t. administered ENK in rats were reported to be at 50 to 100 nmoles. In contrast, significant BP changes were observed at 3.6 nmoles. The absolute doses have to be considered with caution, however, since it is our ex-
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TABLE 4. Summary of Results Obtained With the Injection of Leucine-Enkephalin (Leu-ENK) into the Lateral Brain Ventricle (i.v.t.), into the Fourth Brain Ventricle (i.c.i.), and Intravenously (i.v.) in Rats

<table>
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<tr>
<th>Effects</th>
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<th>i.c.i.</th>
<th>i.v.</th>
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<td>BP ↑</td>
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<td>Effects of naloxone on BP and HR</td>
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<td>inhibition</td>
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<td>“Receptors”*</td>
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<td>μ</td>
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</tr>
<tr>
<td>Effects on BP in SHR-sp</td>
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<td>no difference</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Effects on BP in rats with diabetes insipidus</td>
<td>↓</td>
<td>not studied</td>
<td>not studied</td>
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*Classification merely based on localization and naloxone blockade.

Arrows indicate increase (up) or decrease (down). Results in SHR-sp were compared with age and sex-matched normotensive WKY control rats. Results in diabetes insipidus rats of the Brattleboro strain were compared with Long-Evans rats. BP = blood pressure; HR = heart rate.

experience that unacceptable potency differences exist between different commercial ENK batches. All experiments reported here were performed with one single batch of the respective peptides.

Hemodynamic effects of opioid peptides and morphine have been described previously by other authors. Hemodynamic effects of opioid peptides and morphine have been described previously by other authors. The pentapeptide Leu-ENK appears to be an opioid peptide with almost exclusive pressor properties, while β-endorphin, which consists of 30 amino acids and has a longer half-life, also produced important BP decreases. The adrenaline vaso-depressor and the noradrenaline vasopressor pathways exhibit some analogy to the BP-increasing and decreasing properties of opioid peptides. Both effects can be blocked by naloxone. Whether this explains why naloxone produced no characteristic cardiovascular effects in normotensive and hypertensive animals in this study remains to be investigated. During endotoxin shock hypotension, it has been shown that peripheral opioid peptide receptor blockade can lead to BP increases. Thus, with the various opioid peptides being present in brain areas that are involved in cardiovascular regulation such as the medulla oblongata, mesencephalic nuclei, hypothalamus, median eminence, and amygdaloid nuclei, a complex, partly pressor (e.g., Leu-ENK) and partly depressor (e.g., β-endorphin) peptidergic system of cardiovascular control appears to be emerging.

The opiate receptor antagonist, naloxone, inhibited the BP and HR effects of i.v.- and i.c.i.-administered Leu-ENK. The fact that i.v. Leu-ENK was not counteracted by i.c.i. naloxone. Similar results were obtained by Feldberg and Wei. They found that naloxone inhibited morphine-induced cardiovascular effects i.c.i. only and not i.v.t. It therefore appears that multiple opiate receptors in various brain regions mediate the cardiovascular opioid peptide effects. The naloxone-insensitive receptor sites in the brain ventricles (i.v.t.) could correspond to the δ-receptors, and the i.c.i. and i.v. receptors could correspond to the naloxone-sensitive μ-receptors.

This conclusion of multiple opiate receptors mediating the cardiovascular actions of Leu-ENK is supported by results in SHR-sp. These hypertensive animals exhibited a supersensitivity to Leu-ENK following i.v. and i.v.t., but not following i.c.i. administration of the peptide. In addition, rats with diabetes insipidus showed no increase in BP but, instead, a decrease following i.v.t. Leu-ENK.

We have reported that central ANG II effects could be reduced by beta-adrenergic receptor blockers. This holds true for compounds with and without "nonspecific" membrane effects (unpublished observations). The site of action was clearly shown to be the brain. The possibility of an interaction between brain catecholamines and central peptidergic transmitter systems is now supported by the efficacy of beta-blockers to lower BP and HR responses induced by i.v.t. Leu-ENK, while i.v.-induced increases remained unchanged. These observations may be best explained by peptide effects on brain catecholamines. Clearly, more detailed analyses of central and peripheral nerve activity, effects on hormones, and effects of further pharmacological antagonists of ENK need to be studied for better understanding of the mode of action of ENK on BP.

We have previously formulated the hypothesis that brain peptides participate in central mechanisms of BP regulation by a "central peptidergic stimulation syndrome" which is characterized by a stimulation of a typical pattern of circulating hormones, i.e., ADH, ACTH, and catecholamines. This hypothesis was...
based inter alia on data obtained with angiotensin, namely, 1) that stimulated ANG II biosynthesis in the brain leads to an increased secretion of ACTH, ADH and catecholamines;19, 20 2) that this humoral pattern of increased ADH, ACTH, and catecholamines was prevalent in SHR-sp;21 3) that these rats exhibit a supersensitivity to ANG II;3 4) that the brain renin-angiotensin system is stimulated in these rats;7, 18, 41 and 5) that central ANG II receptor blockade results in a lowering of BP in SHR-sp.22, 42

The present study confirms that brain peptides may be important for cardiovascular control by similar mechanisms as described for ANG II. Thus, ADH appears to be necessary for i.v.t. Leu-ENK BP effects, since diabetes insipidus rats did not respond with BP increases. Bisset et al.13 had previously described the release of ADH by enkephalins. A role of enkephalins in the regulation of vasopressin secretion is also supported by the presence of enkephalin-positive cell bodies in the paraventricular and supraoptic nuclei of the hypothalamus projecting to the posterior pituitary.11

Interactions of enkephalins with brain catecholamines,8, 14, 18 the increase of HR, and the blunting of the vagal component of the baroreceptor reflex as well as the behavioral changes and inhibition of central enkephalin effects by beta-blockers, are in agreement with the assumption of a stimulation of sympathetic tone by Leu-ENK. The roles of ACTH and corticosterone have not been studied in these experiments. As shown for ANG II, however, they could also participate in the BP responses to enkephalin since ACTH has a common high molecular weight precursor with the opioid peptides13 and beta-endorphin is secreted in equimolar quantities together with ACTH into the blood upon stress and painful situations that also lead to BP increases. The increased sympathetic responsiveness, increased ADH and increased ACTH and corticosterone in SHR-sp,42 may thus be caused by central peptidergic stimulation1 and Leu-ENK could contribute to the BP elevation via a central peptidergic mechanism as outlined above.

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