Effects of [D-Ala$^2$]-Methionine-Enkephalin on Blood Pressure, Heart Rate, and Baroreceptor Reflex Sensitivity in Conscious Cats

TOKIHITO YUKIMURA, M.D., GÖNTER STOCK, M.D., HEINRICH STUMPF, THOMAS UNGER, M.D., AND DETLEV GANTEN, M.D., PH.D.

SUMMARY Effects of intracerebroventricular (i.c.v.) injection of [D-Ala$^2$]-methionine-enkephalinamide (DAME) on blood pressure (BP), heart rate, and baroreceptor reflex sensitivity were studied in conscious cats. DAME was administered at doses between 5 and 100 nmoles. Blood pressure and heart rate increased dose dependently. The sensitivity of the baroreceptor reflex was attenuated for 15 to 60 minutes after DAME administration; this was independent of the BP changes. The effects of enkephalin on BP and baroreceptor reflex were abolished by i.c.v. naloxone. DAME caused pathological changes in the electroencephalogram (EEG) characterized by sharp waves in the hippocampus recordings and a loss of theta activity in the electrocorticogram. Behavioral changes were characterized by decreased physical mobility and anxiousness. These behavioral and EEG changes lasted for a longer period of time than the cardiovascular changes; they were also counteracted by naloxone.

It is concluded that DAME produces a centrally mediated vasopressor response and a baroreceptor reflex attenuation and that, with respect to the time course, the effects on the baroreceptor reflex are separated from those on BP behavior and EEG, but not on heart rate. The fact that all effects of enkephalin on the parameters tested in the present experiment were completely antagonized by naloxone suggests that they are mediated by naloxone-sensitive enkephalin brain receptors. (Hypertension 3: 528-533, 1981)

KEY WORDS • enkephalin • naloxone • baroreceptor reflex • blood pressure • heart rate • cat

BRAIN peptides such as angiotensin, substance P, kinins, enkephalins, and endorphins may play a role in central blood pressure (BP) regulation. Among them, the opioids are receiving increasing attention since they have been found present in brain cardiovascular control centers and have produced potent BP effects following central administration apart from their actions on pain sensation and behavior.

In a previous study, it was observed that the effects of leucine enkephalin on the BP of conscious rats were partially inhibited by naloxone if the drugs were administered into the fourth brain ventricle, but not into the third brain ventricle. This indicated that different receptors were mediating the cardiovascular leucine-enkephalin effects. The effectiveness of naloxone on the baroreceptor reflex has not been investigated; the relationship between the effects of enkephalin on BP and heart rate and those on the baroreceptor reflex also is not known, but is of interest in view of the presence of enkephalin-immunoreactive material in the nucleus tractus solitarii, which is the first synapse of the baroreceptor afferents.

In this context, it is important to control the electroencephalogram (EEG) of experimental animals, since epileptic-like spike-wave complexes have been described following central opioid peptides and these could entail cardiovascular responses. It is also known that the hypothalamus, the limbic system, and the cerebral cortex have an important role in the integration of BP control mechanisms and, e.g., baroreceptor reflex modulation.
The purpose of the present experiments was to investigate the effects of the long-lived enkephalin analog [D-Ala²]-methionine-enkephalinamide (DAME) on the BP, heart rate, and baroreceptor reflex in conscious, unrestrained cats. The sensitivity of the baroreceptor reflex was tested every 15 minutes after DAME administration. In addition, the effects of naloxone on the response of the BP, heart rate, and baroreceptor reflex to enkephalin were examined.

Material and Methods

Five female cats weighing 2.5 to 3.5 kg were used in the experiments. They were anesthetized with pentobarbital sodium, and glass-insulated stainless steel electrodes were implanted into the central amygdala and hippocampus using the Horsley-Clarke stereotaxic apparatus, as described. The cortical electrode was implanted into the suprasylvian cortex. A stainless steel cannula was implanted into the lateral venous catheter was placed into the jugular vein. After completion of surgery, the cats were allowed to recover for at least 1 week.

Each cat was given DAME at doses of 5, 10, 25, 50, and 100 nmoles into the brain ventricle, with more than a 3-day interval between each dose. After completion of the first series of experiments, naloxone was injected i.c.v. at a dose of 500 nmoles 3 minutes prior to enkephalin application to test the effects of the opioid antagonist on enkephalin action. To measure the sensitivity of the baroreceptor reflex, the animals were injected intravenously (i.v.) with angiotensin (Hypertensin, Ciba-GEIGY) (0.1 μg), and the increase in pulse interval was plotted against the increase in systolic BP. The sensitivity of the baroreceptor reflex can be estimated by the alteration of this curve. This was tested by i.v. injection of angiotensin 3 times or more before i.c.v. administration of enkephalin, and was repeated every 15 minutes for 2 hours following enkephalin. [D-Ala²]-methionine-enkephalinamide (Lot No. BO53) was purchased from Bioproduct, Belgium.

Results are expressed as means ± SEM. To evaluate statistical differences, Student's paired t test was used. Statistical significance was accepted at the 5% level.

**Results**

**Effects of DAME on Blood Pressure and Heart Rate**

Injection of DAME at doses of 5 to 100 nmoles into the lateral brain ventricle of conscious cats caused dose-dependent increases in systolic BP and heart rate within a few minutes. At the dose of 50 nmoles, systolic BP rose from 143 ± 9 mm Hg to 168 ± 15 mm Hg (p < 0.05), and heart rate increased from 147 ± 6 beats/min to 166 ± 8 beats/min (p < 0.05) 15 minutes after injection. The maximal increase in BP was observed at 15 minutes, but heart rate continued to rise until 30 minutes after injection (table 1, fig. 1). A similar pattern and time course was obtained with the lower doses of enkephalin, and the duration of action was related to the dose injected. No significant rise in BP was caused by 5 and 10 nmoles of DAME, but the heart rate was remarkably increased (fig. 1). The BP fell to control levels after 60 and 90 minutes following 50 and 100 nmoles, respectively. At the dose of 25 nmoles of enkephalin, the BP increase was short-lasting, but the heart rate rose continuously until 30 minutes after the injection (table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Control</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAME 25 nmoles</td>
<td>133 ± 8</td>
<td>154 ± 14*</td>
<td>149 ± 13</td>
<td>144 ± 11</td>
<td>134 ± 13</td>
<td></td>
</tr>
<tr>
<td>+ naloxone</td>
<td>133 ± 11</td>
<td>124 ± 17</td>
<td>133 ± 18</td>
<td>118 ± 14</td>
<td>125 ± 18</td>
<td></td>
</tr>
<tr>
<td>DAME 50 nmoles</td>
<td>143 ± 9</td>
<td>168 ± 15*</td>
<td>163 ± 12</td>
<td>148 ± 11</td>
<td>129 ± 7</td>
<td></td>
</tr>
<tr>
<td>+ naloxone</td>
<td>136 ± 9</td>
<td>150 ± 10</td>
<td>153 ± 9</td>
<td>148 ± 21</td>
<td>146 ± 25</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart rate (beats/min)</th>
<th>Control</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAME 25 nmoles</td>
<td>154 ± 12</td>
<td>182 ± 12*</td>
<td>188 ± 13*</td>
<td>164 ± 10</td>
<td>146 ± 12</td>
<td></td>
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<tr>
<td>+ naloxone</td>
<td>149 ± 24</td>
<td>164 ± 21</td>
<td>158 ± 20</td>
<td>158 ± 27</td>
<td>172 ± 23</td>
<td></td>
</tr>
<tr>
<td>DAME 50 nmoles</td>
<td>147 ± 6</td>
<td>166 ± 8*</td>
<td>193 ± 9*</td>
<td>160 ± 9</td>
<td>142 ± 6</td>
<td></td>
</tr>
<tr>
<td>+ naloxone</td>
<td>144 ± 5</td>
<td>193 ± 29*</td>
<td>180 ± 20*</td>
<td>146 ± 9</td>
<td>149 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM. *p < 0.05.
Effects of Enkephalin on the Baroreceptor Reflex

The increased pulse interval following the elevation of BP produced by i.v. injection of angiotensin was used to estimate the baroreceptor reflex function. After injections of 50 and 100 nmoles of DAME, the baroreceptor reflex was attenuated for 60 minutes (fig. 2). Injection of 25 nmoles of DAME produced a blunting action on the baroreceptor reflex observed at 15 and 30 minutes (fig. 3). Even 5 nmoles of enkephalin blunted the baroreceptor reflex.

Effects of DAME on Behavior and EEG

The EEG changes in the awake cat were characterized by sharp waves in the hippocampus recording and attenuation of theta activity in the electrocortico-gram, lasting more than 2 hours following administration of 50 and 100 nmoles of enkephalin (fig. 4). Increased theta activity in the hippocampus recording was observed in two of five animals.

The behavioral changes were characterized by reduced motility, reduced spontaneous activity, and anxiously. The cats were reactive to sound; no catatonia occurred. These enkephalin-induced changes in behavior and EEG lasted for more than 2 hours and were dose-dependent.

Effects of DAME in Naloxone-Treated Animals

Naloxone (500 nmoles) injected i.c.v. 3 minutes prior to the administration of 25 and 50 nmoles of DAME completely blocked all cardiovascular responses to 25 nmoles of DAME (table 1, fig. 5). Naloxone also blocked the BP responses, but not the heart rate increases or baroreceptor reflex attenuation at the higher dose of 50 nmoles (table 1). Naloxone completely abolished the changes in behavior and EEG at all doses of DAME tested.

Discussion

DAME is a synthetic analog of methionine enkephalin and is metabolically more stable than the natural peptide. The present experiments were carried out to investigate the effects of centrally administered DAME on the cardiovascular system including baroreceptor reflex, behavior, and EEG in conscious, unrestrained cats.

Arterial BP increased in dose-dependent increments when the compound was administered into the lateral brain ventricle. The results supply further evidence that DAME produces a vasopressor response via a central mechanism. The baroreceptor reflex was attenuated for 15 to 60 minutes after DAME administration.

Involvement of enkephalins in central mechanisms of BP regulation has been proposed. Moreover, changes have been reported in the arterial BP and heart rate following central administration of opioid peptides and receptor antagonists; these may be pressor, depressor, or biphasic in nature depending on the peptide used, site of administration, animal species, its state of consciousness, and experimental protocol. In certain forms of shock, circulating opioid peptides (probably endorphins) have a BP lowering effect; consequently, naloxone leads to BP elevation in such situations. This hypotensive action of long-chain endorphins was shown to be mediated by naloxone-sensitive \( \mu \) receptors that may be located in the medullary vasomotor center. In contrast, leucine enkephalin had only pressor effects which, in the conscious rat, are mainly mediated by naloxone-insensitive \( \delta \)-receptors located around the third brain ventricle, possibly in the hypothalamic area. In anesthesia, these centrally elicited BP increases can be blunted or even reversed to hypotensive effects, as has been reported for other brain peptides.

The fact that naloxone could counteract the inhibitory effect of DAME on the baroreceptor reflex as well as on pressor and heart rate responses suggests...
that, in the cat, the opioid peptide elicits its effect via opioid receptor stimulation in the brain. Since it has been reported that, in rats, naloxone inhibits the effects of enkephalin following intracisternal and intravenous, but not intracerebroventricular injection, it is possible that there may be some species differences with regard to localization and character of opioid receptors.

In spite of the large increase in BP following i.c.v. administration of DAME, no reflex bradycardia was observed, which may be due to blunting of the baroreceptor reflex by the peptide itself. We therefore tested the baroreceptor reflex sensitivity by plotting the pulse interval elongation of each beat against the increase in systolic BP following several doses of enkephalin with and without opioid receptor antagonist. The previously reported inhibition of the baroreceptor reflex was confirmed. In addition, it was found that the baroreceptor reflex sensitivity was blunted after low doses of enkephalin that did not have any pressor effect. The duration and extent of the baroreceptor reflex depression was dose-related.

These results indicate that DAME inhibited the vagal component of the baroreceptor reflex. Since enkephalins have been shown to inhibit synaptic transmission in the central and peripheral nervous system, this may be explained by an inhibitory effect of DAME on neural transmission.

Our observation that there was a time lag between maximal increase in BP and heart rate suggests a different mode of action of the peptide on BP and heart rate. This assumption is favored by the following results: First, at the dose of 5 and 10 nmoles, tachycardia was observed without any significant
change in BP. The stimulatory action of DAME on heart rate was more pronounced. Second, naloxone blunted the BP only, not the heart rate responses to enkephalin at the dose of 50 nmoles, although it completely counteracted all actions following 25 nmoles of enkephalin. Finally, the baroreceptor reflex sensitivity was always attenuated with changes in conjunction with heart rate, but not with BP. The increase in BP itself had no influence on the baroreceptor reflex, as was reported by Smyth et al.1 It could also be that the increase in heart rate, brought about by DAME, feeds back on the baroreceptor sensitivity. In this case, the effect of DAME on the baroreceptor reflex would have to be considered indirect. However, previous studies (unpublished data) have indicated that heart rate increases of up to 250 beats/min did not attenuate the baroreceptor reflex to the degree reported here after DAME administration.

Important changes were observed in the behavior and EEG of the animals following DAME. The EEG recorded from the hippocampus showed sharp waves, which typically originate from hypothalamus and amygdala. This is of interest because of the importance of these brain areas for central cardiovascular

**FIGURE 4.** Electroencephalogram (EEG) from central amygdala, hippocampus, and cerebral cortex before and after intracerebroventricular (i.c.v.) injection of DAME (100 nmoles).
control.\textsuperscript{39, 50} Episodic spike-wave complexes and epileptic seizures reported previously\textsuperscript{4} were not observed in these experiments. Changes in EEG and behavior were completely antagonized by pretreatment with i.c.v. naloxone. But the effects on behavior and EEG following DAME had a different dose-effect relationship and were longer lasting compared to the cardiovascular changes.

The significance of central peptidergic stimulation for BP control in normotensive and hypertensive animals has been studied best for angiotensin.\textsuperscript{1, 3, 4, 21, 22} Recently, enkephalins,\textsuperscript{1} substance P,\textsuperscript{22} and kinins\textsuperscript{4} have also been shown to increase BP centrally. The mechanism by which they bring about their cardiovascular effects may be different for each peptide, but the stimulation of pituitary hormones, e.g., ADH and ACTH, and an increase of sympathetic tone appear to be a common feature of central peptidergic stimulation.\textsuperscript{1} An involvement of increased sympathetic tone in the enkephalin-mediated pressor responses is supported by the fact that beta-adrenergic blockers inhibit the leucine-enkephalin-elicited BP increases,\textsuperscript{9} and that cardiac contractility is enhanced by central enkephalins. In addition, we have now reported that the vagal component of the baroreceptor reflex, which would normally counterregulate BP increases, is markedly attenuated by enkephalins. This is a further possible mechanism by which enkephalins could interfere in central mechanisms of cardiovascular control and contribute to the BP elevation.

Acknowledgments

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References

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