Vasopressin Release Does Not Contribute to Pressor Action of Enkephalin in SHR

ROBIN W. ROCKHOLD, PH.D., JOAN T. CROFTON, B.S., AND LEONARD SHARE, PH.D.

SUMMARY The effects of injection of a peptidase-resistant analog of methionine-enkephalin, [D-Ala\(^1\)]-methionine-enkephalin, on blood pressure (BP), heart rate, and vasopressin release were studied in spontaneously hypertensive rats (SHR). Intravenous injection of [D-Ala\(^1\)]-methionine-enkephalin (DAME) increased BP in both SHR and normotensive Wistar-Kyoto (WKY) controls, with a significantly greater increase in hypertensive rats. Intracerebroventricular injection of DAME produced a biphasic increase in BP and an increase in heart rate in both groups. The initial pressor effect was significantly greater in the SHR. Plasma vasopressin levels in SHR were depressed relative to both untreated hypertensive rats and animals given vehicle control injections. Intravenous pretreatment with a vasopressin vasopressor antagonist, [1-(\(\beta\)-mercaptoglu-taramino)pentapeptide-acid]- \(\beta\)-(o-methyl)tyrosine-\(\beta\)-arginine-vasopressin, did not block either component of the central enkephalin response in hypertensive rats. These data indicate that central enkephalin injection does not release vasopressin and that SHR are hyperresponsive to enkephalin. It is concluded that pressor systems other than that of vasopressin mediate the enkephalin-induced cardiovascular effects. (Hypertension 3: 410-415, 1981)

KEY WORDS • vasopressin • enkephalin • blood pressure • spontaneously hypertensive rats • antidiuretic hormone

It has been shown that administration of opioid peptides into the central nervous system can elicit highly variable changes in blood pressure (BP) and heart rate.\(^1\)\(^-\)\(^5\) An indication that these neuropeptides may be important endogenous regulators of vascular and myocardial tone is the finding that opioid peptides, particularly the pentapeptides leucine and methionine-enkephalin, are found in high concentrations in cell bodies and nerve terminals within neural structures known to be involved in cardiovascular control. Of specific interest is the localization of enkephalin in catecholamine-containing nuclei, such as the solitario-vagal complex,\(^4\) the arcuate nucleus,\(^4\) and the nucleus interstitialis of the stria terminalis,\(^4\)** as well as in magnocellular neurosecretory nuclei,\(^4\)** and in the posterior pituitary.\(^7\)

Recently it has been suggested that, in animal models of genetic hypertension, there may be correlations between the hypertension and changes in the levels of the enkephalins in autonomic ganglia.\(^8\) Further evidence implicating enkephalins in hypertension was reported by Schaz et al.,\(^*\) who found enhanced pressor responses in stroke-prone spontaneously hypertensive rats (SHR), compared to Wistar-Kyoto rats (WKY), to intracerebroventricular (icv) infusion of leucine-enkephalin. The mechanism for this enhanced pressor responsiveness is unclear. Release of an antidiuretic factor\(^10\) (presumably arginine vasopressin) has been reported following intracerebroventricular enkephalin administration. Vasopressin is an extremely potent vasoactive hormone,\(^11\) and has been shown\(^12\) to exist in elevated levels in plasma, urine, and pituitary glands of SHR. The demonstration that rats homozygous for hereditary hypothalamic diabetes insipidus responded with BP decreases to central leucine-enkephalin infusion,\(^13\) a response opposite that seen in rats with intact neurohypophysial function, suggested that vasopressin re-
lease plays a significant role in the enhanced pressor responsiveness of SHR to enkephalin. Experimental reports have described both increases and decreases in plasma vasopressin levels following administration of opioid peptides.

We have employed a peptidase-resistant analog of methionine-enkephalin, [D-Ala^1]-methionine-enkephalin in studies designed to determine the role of vasopressin in the cardiovascular response of SHR to enkephalin.

**Methods**

**General Procedures**

Male SHR and WKY (WKY, Taconic Farms, Inc.) were used when they were 11 to 16 weeks of age, after being housed singly in wire mesh cages in a temperature-controlled (22°C-24°C) and light-controlled (12 hours light; 12 hours dark) room, and given commercial rat chow (Purina Laboratory Chow) and tap water ad libitum.

All surgical procedures were performed under ether anesthesia. Polyethylene cannulae (PE-20) were inserted into the left lateral cerebral ventricle following placement of the rats in a stereotaxic instrument so that the horizontal plane between the sutures bregma and lambda was perpendicular to the vertical axis. Coordinates relative to bregma were: posterior 0.5 mm; lateral 1.3 mm; and 5.0 mm below the dorsal skull surface. Two stainless steel anchoring screws were placed into the parietal bones, and dental acrylic secured the cannulae in place. Penicillin (30,000 U, Flocillin) was given intramuscularly following surgery. The right femoral artery and vein were catheterized following a recovery period of a minimum of 5 days and 12-18 hours before an experiment. The polyethylene catheters (PE-50 and PE-10 respectively) were exteriorized at the nape of the neck, filled with heparinized saline, and sealed until use. Penicillin was again administered. Verification of placement of the cannula into the lateral ventricle was accomplished by icv injection of a vegetable dye, removal of the brain, and visual inspection of staining in the lateral, third, and fourth ventricles.

The BP and heart rate were monitored while the animals were conscious and unrestrained in plastic shoebox cages. The BP was measured with Statham P23 Gb or P23 ID transducers and displayed on a Gould Brush 2400 recorder. Heart rate was determined by manually counting expanded tracings of the BP record at 5-minute intervals.

**Enkephalin Dose-Response Experiments**

Following a 30-minute equilibration period, intravenous bolus injections (0.2 ml) of 0.85, 8.5, and 85.0 nmoles [D-Ala^1]-methionine-enkephalin (DAME; Peninsula Laboratories, Inc.) were given sequentially to each animal. The interval between injections averaged approximately 1 hour. When BP and heart rate had returned to preinjection levels following the last intravenous injection, a second (15-30 minute) equilibration period was begun. Upon completion of this period, icv injections were given in a total volume of 15 μl (10 μl drug and 5 μl vehicle flush) over a 30-second period using a hand-driven Hamilton microliter syringe. Artificial cerebrospinal fluid adjusted with mannitol (ACSF) to equal the calculated osmotic strength of the most concentrated DAME solution (85.0 nmoles) was injected icv, and then was followed, at a minimum of 1-hour intervals, by 0.85, 8.5, and 85.0 n mole doses of DAME.

**Plasma Vasopressin Measurements**

The rats were prepared as described above, with the exception that a PE-50 polyethylene catheter was inserted in the femoral vein. SHR received no treatment, neither icv injection of 85.0 nmoles DAME, nor icv ACSF. Blood samples were taken at time 0 (following a 30-minute equilibration period), or at 2 minutes or 25 minutes following icv injection of ACSF vehicle or DAME. Blood sampling was performed by manual withdrawal (during 75 seconds) of 2.2 ml of arterial blood into a heparinized plastic syringe, while an equal volume of a plasma expander (3% polyvinylpyrrolidone in artificial extracellular fluid) was simultaneously infused intravenously. Blood was placed in iced heparinized plastic tubes, and plasma was obtained by centrifugation at 4°C. Vasopressin levels were determined following extraction with Sep-Pak C^18 cartridges (Waters Associates) by a sensitive and specific radioimmunoassay. The U.S.P. Posterior Pituitary Reference Standard was used as the vasopressin standard. The recovery of 10.5 μg of vasopressin from 1 ml aliquots of rat plasma averaged 78.3% ± 1.6% (n = 10). Plasma sodium and potassium concentrations and osmolality were measured with an IL 343 flame photometer (Instrumentation Laboratory, Inc.) and an Osmette A osmometer (Precision Scientific, Inc.) respectively. Hematocrit was determined by a microcapillary method.

**Vasopressin Antagonist Experiments**

Intravenous injections (5 μg/kg) of a long-acting antagonist, [1-(β-mercapto-β,β-cyclopentamethyl-enepropionic acid),2-(0-methyl) tyrosine]arginine-vasopressin, of the pressor action of vasopressin or vehicle (0.9% NaCl, 0.1% bovine serum albumin, 0.03% glacial acetic acid) were given to SHR. Fifteen minutes following these treatments, 85.0 nmoles of DAME was injected icv as described above. The BP, heart rate, and general behavior were observed.

**Statistics**

To correct for nonhomogeneity of variance, a logarithmic transformation was performed on all vasopressin data. Analysis of variance (one- or two-way for appropriate groups) was performed on experimentally derived values. Suitable post hoc tests (Newman-Keuls or multiple t tests) were performed if significant interactions were found. Means ± 1 SEM are presented.
Results

[D-Ala₁]-methionine enkephalin, when administered as intravenous bolus injections (0.85, 8.5, and 85.0 nmole), increased mean arterial blood pressure (MAP) in both SHR and WKY. The BP responses were characterized by a rapid monophasic increase which returned to control levels within 1 minute (table 1). A significantly (p < 0.005) greater pressor effect was noted in SHR when compared to WKY after the 85.0 nmole dose.

Injection of DAME into a lateral brain ventricle produced biphasic increases in MAP and an increase in heart rate. Figure 1 demonstrates the patterns of change in MAP following icv injection of 85.0 nmole of DAME. An initial rise, with a peak after 2 minutes after injection, was found to be significantly greater (p < 0.005) in the SHR than in the WKY. A subsequent plateau phase of the BP response was noted to be similar in the SHR and WKY, with maximal values at approximately 25 minutes after injection. Figure 2 presents the dose-response relationship for increases in MAP in both strains at 2 and 25 minutes after injections. Pre-drug injection values of MAP for vehicle, 0.85, 8.5, and 85.0 nmole doses were 157 ± 4*, 158 ± 4*, and 159 ± 4* mm Hg in WKY and 119 ± 3, 120 ± 3, and 119 ± 3 mm Hg in SHR. Consistently greater responsiveness was noted in SHR (2-6 times that seen in WKY) at the 2 minute time point. No differences were noted between groups in the responses at 25 minutes. A further difference was found between the two strains. The greatest increase in MAP in the WKY following the 85.0 nmole dose occurred during the plateau phase (22 ± 2 mm Hg). This was significantly greater than the initial pressor response of 14 ± 2 mm Hg (p < 0.05). The greatest increase in the SHR occurred during the initial phase. A marked tachycardia was noted in both strains, with maximal increases 25 minutes after injection. The maximal increase in heart rate was greater in WKY than in SHR (140 ± 15 vs 86 ± 18 beats/min,

<table>
<thead>
<tr>
<th>Dose (nmole)</th>
<th>Initial Max (mm Hg)</th>
<th>Max Initial Max (mm Hg)</th>
<th>No.</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>157 ± 4*</td>
<td>2 ± 2</td>
<td>(8)</td>
<td>119 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>0.85</td>
<td>158 ± 4*</td>
<td>10 ± 3</td>
<td>(11)</td>
<td>118 ± 3</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>8.50</td>
<td>159 ± 4*</td>
<td>30 ± 3</td>
<td>(11)</td>
<td>120 ± 4</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>85.00</td>
<td>157 ± 5*</td>
<td>45 ± 2†</td>
<td>(11)</td>
<td>118 ± 4</td>
<td>34 ± 2</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of animals in each group.

* p < 0.001 when compared to WKY.
† p < 0.005 when compared to WKY.

p < 0.005). However, the initial heart rate was significantly lower in WKY (323 ± 9 vs 364 ± 14 beats/min, p < 0.05).

A "wet-dog" shaking phenomenon was associated with the initial pressure response to icv DAME. Typically, shaking commenced after the BP began to rise and continued for as long as 5 minutes after injection. At the 85.0 nmole dose, 90% (9 of 10) SHR responded, while 36% (4 of 11) WKY shook; at 8.5 nmole, 64% (7 of 11) SHR responded, as opposed to 18% (2 of 11) WKY. No animals in either groups responded in this fashion following injection of 0.85 nmole of DAME or vehicle.

![Figure 1](http://hyper.ahajournals.org/)

** Figure 1. Change in mean arterial blood pressure (ΔMBP) (mm Hg) in conscious SHR and WKY following injection of [D-Ala₁]-methionine enkephalin (DAME) (85.0 nmole, icv). Asterisks indicate statistically significant differences (p < 0.005) relative to vehicle control.**

![Figure 2](http://hyper.ahajournals.org/)

** Figure 2. Upper graph: Maximum changes in mean arterial blood pressure (ΔMBP) in SHR and WKY 2 minutes following icv injections of vehicle, 0.85, 8.5, and 85.0 nmole of [D-Ala₁]-methionine-enkephalin (DAME). Asterisks indicate statistically significant differences between the groups. Lower graph: Maximum changes in MAP (mm Hg) in SHR and WKY 25 minutes following icv injections of vehicle, 0.85, 8.5, and 85.0 nmole of DAME.**
Conscious, unrestrained, and chronically cannulated SHR had plasma vasopressin levels of 2.33 ± 0.14 μU/ml (fig. 3). The icv injection of ACSF did not significantly alter the plasma vasopressin concentration at either the 2- or 25-minute sampling times (fig. 3). Injections of DAME (85.0 nmoles) decreased plasma vasopressin to 72% of vehicle control at 2 minutes and to 34% at 25 minutes; only the latter change was statistically significant (p < 0.005). Plasma osmolality rose in DAME-treated SHR from 292 ± 0.7 mOsm/kg H₂O at time 0 to 296 ± 1.7 mOsm/kg H₂O (p < 0.05) at 2 minutes, and 296 ± 0.9 mOsm/kg H₂O (p < 0.05) at 25 minutes. These values were significantly greater than those seen in vehicle-treated SHR at 2 minutes (292 ± 0.9 mOsm/kg H₂O, p < 0.05) and 25 minutes (293 ± 0.7 mOsm/kg H₂O, p < 0.05). However, neither hematocrit nor plasma sodium and potassium concentrations were significantly changed in drug-treated SHR. The pattern of pressor responses in those experiments in which plasma vasopressin levels were measured was not significantly different from the BP effects seen in SHR during the dose-response experiment (following icv injection of 85.0 nmoles of DAME). The MAP was not significantly affected by blood sampling, although heart rate was slightly but significantly increased in all groups. There were no significant differences in this response between times of sampling or between vehicle- and drug-treated groups, so the values were pooled. A mean increase in heart rate of 37 ± 6 beats/min (n = 44) was noted following the sampling procedure.

The intravenous injection of 5 μg/kg of a vasopressin vasopressor antagonist ([(1-β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(0-methyl)tyrosine]arginine-vasopressin) in normotensive female Sprague-Dawley rats virtually abolished the pressor response to intravenous injection of arginine vasopressin (6.25-50.0 μg/kg) for a period of at least 1 hour, while the pressor responses to intravenous injections of norepinephrine (0.25-2.0 μg/kg) or angiotensin II (100 ng/kg) were not significantly altered. Similar injections of the antagonist did not alter resting MAP in conscious SHR (−7.2 ± 2.5 mm Hg) when compared to vehicle-treated rats (−7.1 ± 1.8 mm Hg), while reducing the pressor response to intravenous injection of arginine vasopressin to 20% of control (table 2). Intracerebroventricular injection of DAME (85.0 nmoles) in SHR 15 minutes following antagonist pretreatment produced BP changes which were not significantly different from those noted with DAME following intravenous injection of the vehicle for the antagonist (fig. 4). Similarly, the tachycardic effect to icv DAME was not blunted, with a maximum increase in vehicle-treated SHR of 106 ± 14 beats/min vs 87 ± 11 beats/min in antagonist-treated rats.

**TABLE 2. Effect of Intravenous Injection of [1-(β-Mercapto-β,β-Cyclopentamethylenepropionic Acid),2-(0-Methyl) Tyrosine]Arginine-Vasopressin (5 μg/kg) or Vehicle on the Pressor Response to Arginine Vasopressin (25 ng/kg, i.v.) in SHR**

<table>
<thead>
<tr>
<th>BP before (mm Hg)</th>
<th>BP after (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antagonist</td>
<td>Vehicle</td>
</tr>
<tr>
<td>40 ± 2 (9)</td>
<td>35 ± 3 (8)</td>
</tr>
<tr>
<td>8 ± 3* (9)</td>
<td>33 ± 5 (8)</td>
</tr>
</tbody>
</table>

The initial injections of arginine vasopressin were given 15 minutes before antagonist injection, while the second vasopressin dose was given 75 minutes later (60 minutes following antagonist injection). Numbers in parentheses indicate the number of animals in each group.

*p < 0.001.

**Figure 3. Plasma concentrations of arginine vasopressin (μU/ml) in SHR at time zero (no treatment) and following injection of [D-Ala²]-methionine-enkephalin (DAME) (85.0 nmoles, iv) or ACSF vehicle (15 μl, iv). Scale on abscissa indicates time in minutes following iv injection. Asterisks indicate statistically significant differences between the groups. Numbers in parentheses indicate number of animals in each group.**

**Figure 4. Changes in mean arterial pressure (ΔBP) (mm Hg) in SHR following injection of [D-Ala²]-methionine-enkephalin (DAME) (85.0 nmoles, iv) 15 minutes after iv injection of either [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(0-methyl)tyrosine]arginine-vasopressin (d(CH₃)Tyr(Me)AVP, 5 μg/kg) or vehicle.**
Discussion

Our results demonstrate that a long-acting analog of methionine-enkephalin, [D-Ala²]-methionine enkephalin, can markedly alter cardiovascular function. Enkephalin administration has been reported to cause release of vasopressin. It has been postulated that this neuroendocrine mechanism and/or an increase in sympathetic tone might be responsible for the observed enhancement of pressor responsiveness in SHR and, indeed, may be related to the genesis or maintenance of high BP in this hypertensive model.

However, we could find no evidence for increased vasopressin release following central enkephalin in SHR. On the contrary, plasma vasopressin levels fell following icv DAME. These decreases were noted even though plasma osmolality was significantly elevated in DAME-treated SHR. In addition, the lack of alteration of central enkephalin-induced cardiovascular effects following treatment with a vasopressin vasopressor antagonist clearly indicates that increased vasopressin release does not contribute to the pressor response to icv DAME in SHR. It is possible that the vasomotor effects of central DAME are due to increases in sympathetic nerve traffic. The marked increase in heart rate, in the face of a rise in MAP, supports this conclusion. There is evidence that SHR possess a higher basal sympathetic tone than WKY. However, at this time, it is not possible to exclude a potential contribution from other vasopressor systems, notably the renin-angiotensin system.

The lack of difference between SHR and WKY in the magnitude of the plateau phase of the pressor response suggests that the enhanced pressor responsiveness of SHR to icv DAME is not the result of altered peripheral vascular reactivity, as proposed by others. On the other hand, this mechanism may well contribute to the enhanced pressor responsiveness of SHR following intravenous DAME injection.

At the present time, no firm conclusion can be reached concerning the cause of the decrease in BP seen in DI rats following central leucine-enkephalin infusions. However, since the DI rat is known to exhibit deficits in anterior pituitary function, in central opioid systems, and in central angiotensin II immunoreactive material, it is quite likely that defects other than the lack of synthesis and release of vasopressin contribute to the anomalous reaction of this rat strain to enkephalin.

The observed reduction in the plasma concentration of vasopressin in SHR may be explained either by a reflex response to elevated arterial BP or by a direct action of the opioid on the hypothalamic-neurohypophysial axis. Arterial and myocardial mechano-receptors constitute one of the major regulatory systems for vasopressin secretion, and the increases in MAP due to enkephalin would be expected to suppress vasopressin release. In view of the pronounced cardiovascular effects produced by the opiates and the opioid peptides, the failure to monitor arterial pressure may contribute to the diverse nature of the results which have been reported concerning vasopressin release and opioids.

A direct action of opioids on the regulation of vasopressin secretion is supported by recent evidence, in vitro, which indicates that opioids may act directly on the neurohypophysis to suppress vasopressin secretion. Studies conducted in vivo also suggest that central opiate receptors exert an inhibitory influence on vasopressin release. In one study, a biphasic effect of central morphine injections on plasma vasopressin concentration was observed, the most pronounced effect being that of a reduction in plasma levels of vasopressin. It is possible that both direct and baroreceptor reflex-mediated mechanisms are involved in the alterations in vasopressin secretion caused by opioid peptides and the opiates.

The enkephalin-induced antidiuretic effect first observed by Bisset et al. remains intriguing. Nonetheless, our studies and others make it likely that this action was not due to an increase in the plasma concentration of vasopressin. Huidobro-Toro et al. have indicated that the renal effects of opioid peptides are not those expected to result from vasopressin's actions. Since urinary electrolyte excretion decreased in conjunction with urinary volume, these authors concluded that the antiuretic seen with opioid peptides and morphine may reflect changes in central renal regulatory mechanisms.

In conclusion, our investigation provides firm evidence that the cardiovascular changes resulting from central enkephalin administration are not associated with, nor caused by, increased secretion of vasopressin. Additionally, it is suggested that the enhanced pressor responsiveness of SHR to central enkephalin injection results from the activation of a pressor system other than vasopressin, possibly the sympathetic nervous system.

Acknowledgments

The authors gratefully acknowledge the generous donation of the vasopressin antagonist by Dr. M. Manning of the Department of Pharmacology, College of Physicians and Surgeons, Biochemistry, Medical College of Ohio, and Dr. W. H. Sawyer, Department of Pharmacology, College of Physicians and Surgeons, Columbia University. The authors are indebted to Randall Cheshire and Cynthia Allen for their expert technical assistance.

References

Vasopressin release does not contribute to pressor action of enkephalin in SHR.
R W Rockhold, J T Crofton and L Share

doi: 10.1161/01.HYP.3.4.410

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/3/4/410

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org//subscriptions/