Central peptidergic stimulation in blood pressure control: role of enkephalins in rats

T. YUKIMURA, T. UNGER, W. RASCHER, R. E. LANG AND D. GANTEN
Department of Pharmacology, University of Heidelberg, and German Institute for High Blood Pressure Research, Heidelberg, Germany

Summary
1. The central blood pressure effects of the enkephalin analogue, D-Ala²-Met-enkephalin were investigated in normotensive rats and in spontaneously hypertensive rats.
2. In conscious Wistar rats, injection of 1.7, 17 and 170 nmol of D-Ala²-Met-enkephalin into the lateral brain ventricle produced dose-dependent blood pressure increases of 5.3 ± 1.8, 10.5 ± 2.8 and 15.3 ± 3.0 mmHg respectively. In contrast, in α-chloralose-anaesthetized rats, the same doses of the enkephalin analogue produced dose-dependent blood pressure falls of -8.9 ± 1.9, -11.1 ± 5.2 and -19.8 ± 8.4 mmHg.
3. The pressor responses to 170 nmol of D-Ala²-Met-enkephalin were blocked by intracerebroventricular pretreatment with the opiate receptor antagonists diprenorphine and naloxone at doses of 10–500 nmol. The depressor effects under anaesthesia were blocked by diprenorphine (10–100 nmol) but not by naloxone.
4. Spontaneously hypertensive rats showed enhanced pressor responses to centrally applied enkephalin when compared with normotensive rats.
5. Central injection of diprenorphine alone produced an initial short blood pressure increase followed by a long-lasting blood pressure fall, which was significantly greater in spontaneously hypertensive rats than in normotensive rats.
6. The results indicate that the central blood pressure effects of enkephalins depend on the state of consciousness and are mediated through naloxone- and diprenorphine-sensitive opioid receptors.
7. The increased sensitivity to centrally applied enkephalins and the depressor responses to the intracerebroventricular injection of diprenorphine suggest a central enkephalinergic contribution to the maintenance of hypertension in spontaneously hypertensive rats.

Key words: diprenorphine, enkephalins, naloxone, opiate antagonist.

Abbreviations: DAME, D-Ala²-Met-enkephalin; i.c.v., intracerebroventricularly.

Introduction
The regional distribution of enkephalins in the brain has been shown to be partly identical with brain nuclei involved in cardiovascular regulation [1, 2]. This indicates that opioid peptides could play a role in central mechanisms of blood pressure control. Some investigators have reported that centrally applied opiates or opioid peptides increase arterial blood pressure [3–5] but others have observed blood pressure decreases [6–9]. These results were obtained partly in conscious and partly in anaesthetized animals.

In the present experiments, we studied the central blood pressure responses to D-Ala²-Met-enkephalin (DAME) in both conscious and α-chloralose-anaesthetized animals. The synthetic long-lived enkephalin analogue was injected intracerebroventricularly (i.c.v.), into the lateral brain ventricle, in normotensive and spontaneously hypertensive rats. The specificity of the blood pressure effects was tested by means of the opiate receptor antagonists naloxone and diprenorphine. These substances were also used to inhibit endogenous enkephalins.

Materials and methods
Male Wistar rats weighing 200–250 g were housed individually under identical conditions of room temperature and humidity. Adult male and female spontaneously hypertensive rats of the stroke-prone strain (SHRSP), bred in Heidelberg since 1975, and age- and sex-matched normo-
tensive Wistar–Kyoto (WKY) rats were used. A polyethylene cannula (PP 20) was stereotaxically implanted into the lateral brain ventricle for the intracerebroventricular injections at least 1 week before the acute experiment. The following co-ordinates were used: 0.5 mm posterior and 1.3 mm lateral from the bregma and 5.0 mm ventral from the surface of the skull. One day before the experiment, a polyethylene catheter (PP 10 connected to PP 50) was placed in the abdominal aorta through the femoral artery. The catheter was tunneled under the skin and exteriorized at the scruff of the neck. Arterial blood pressure and heart rate were recorded through the arterial catheter on to a microsyringe. Each animal was given only one volume of 10 pl injected with a Hamilton syringe. Diprenorphine was injected i.c.v. at doses of 10 and 100 nmol to investigate the effect of central blockade of endogenous opioid peptides. In conscious rats, injections of 1.7, 17 and 170 nmol of DAME into the lateral brain ventricle produced dose-dependent blood pressure increases of 5.3 ± 1.8, 10.5 ± 2.8 and 15.3 ± 3.0 mmHg respectively. An initial short peak was followed by a second long-lasting increase which had its maximum 15–20 min after the injection, and lasted about 30 min at the highest dose injected. Behavioural changes were characterized by loss of spontaneous mobility without catatonia and lasted more than 1 h at the dose of 170 nmol.

In α-chloralose-anaesthetized rats, the same doses of DAME caused dose-dependent blood pressure falls of −8.9 ± 1.9, −11.1 ± 5.2 and −19.8 ± 8.4 mmHg. The blood pressure decreases were characterized by a long-lasting plateau phase after the initial fast blood pressure drop. Maximum decreases were seen at 8–10 min. Blood pressure returned to its basal level about 1 h after injection.

**Intracerebroventricular injections of DAME after pretreatment with opiate receptor antagonists**

Diprenorphine and naloxone were injected i.c.v. 3–5 min before 170 nmol of DAME. In diprenorphine-treated conscious animals the blood pressure response to 170 nmol of DAME were reversed to blood pressure falls. Pretreatment with naloxone did not counteract the depressor responses to DAME given i.c.v. In α-chloralose-anaesthetized rats, the depressor responses to DAME given i.c.v. were blunted with the lowest dose and completely prevented with the highest dose of diprenorphine. In contrast, pretreatment with 10 and 100 nmol of naloxone did not counteract the depressor responses to DAME given i.c.v.

**Intraventricular injections of DAME and opiate antagonists in spontaneously hypertensive rats**

Conscious SHRSP showed markedly enhanced pressor responses to DAME injected i.c.v. when compared with WKY rat controls. At 170 nmol the respective blood pressure changes were 24.6 ± 3.2 vs 12.0 ± 3.2 mmHg (P < 0.05).

The opiate receptor antagonist diprenorphine given alone i.c.v. at a dose of 100 nmol produced an initial short blood pressure increase followed by a long-lasting blood pressure fall; this was markedly greater in SHRSP than in WKY rats (−18.3 ± 3.0 vs 6.2 ± 2.0 mmHg; P < 0.01) (Fig. 1). After a smaller dose of diprenorphine (10 nmol i.c.v.) blood pressure showed a slight fall in SHRSP (−4.3 ± 1.4 mmHg) without the initial increase.
Central blood pressure effects of opiate antagonist

Discussion

Our results demonstrate that central administration of DAME produces blood pressure increases as well as blood pressure decreases depending on whether the animals were conscious or anaesthetized. Thus the central blood pressure effects of enkephalins clearly depend on the state of consciousness. This could possibly help to explain some of the discrepancies in the literature concerning the central cardiovascular effects of morphinomimetic substances [4, 6–10].

The fact that both opiate receptor antagonists, naloxone and diprenorphine, counteracted the central pressor responses to DAME in conscious animals suggests that this opioid peptide produces its effects through stimulation of specific opiate receptors in the brain. In α-chloralose-anaesthetized rats, however, the depressor responses to DAME injected i.c.v. could only be prevented by diprenorphine and not by naloxone. These findings suggest an involvement of different subtypes of opiate receptors in mediating the central cardiovascular actions of opioid peptides, the exact nature of which remains to be established [4, 11–13].

In the SHRSP, a supersensitivity to centrally administered DAME was observed. Similar results have been reported previously with leucine-enkephalin and methionine-enkephalin [4] but also with other non-opioid brain peptides such as angiotensin [14], bradykinin and substance P [15]. An involvement of these peptides in the maintenance of high blood pressure in SHRSP has been claimed from biochemical and pharmacological evidence [16]. Thus the inhibition of brain angiotensin-converting enzyme with captopril [17] or blockade of central angiotensin receptors with saralasin and other angiotensin antagonists [18] has led to blood pressure falls in SHRSP but not in normotensive rats.

Our finding reported here of a marked reduction of blood pressure in SHRSP after the central administration of diprenorphine provides further evidence that not only a central inhibition of the renin–angiotensin system but also the blockade of brain opiate receptors can lower blood pressure in this type of experimental hypertension. This raises the possibility of opioid peptides in the brain contributing to the maintenance of elevated blood pressure in SHRSP in addition to the brain renin–angiotensin system, and lends further support to the concept of central peptidergic stimulation being a possible pathogenetic factor in hypertension [16].

Acknowledgments

These studies were supported by the Deutsche Forschungsgemeinschaft within the SFB 90 Cardiovasculäres System.
References


