*Br. J. Pharmac.* (1983), **80**, 405–408

# Captopril potentiates the vasodepressor action of Met-enkephalin in the anaesthetized rat

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**1** The transient vasodepressor action of Met-enkephalin (10–80 $\mu g\,kg^{-1}$, i.v.) in anaesthetized rats was significantly potentiated by the angiotensin-converting enzyme inhibitor, captopril (2 $mg\,kg^{-1}$, i.v.); at this dose, it failed to modify the transient vasodepressor action of the non-specific vasodilator, nitroprusside (2.5, 5.0, 10 $\mu g\,kg^{-1}$, i.v.).

**2** Captopril (2 $mg\,kg^{-1}$, i.v.) caused a slow, progressive fall in the blood pressure of anaesthetized spontaneously hypertensive (SH) rats when compared to vehicle-treated controls. Pretreatment with naloxone (1.5 $mg\,kg^{-1}$, i.v.) 30 min earlier failed to alter significantly the hypotensive action of captopril in anaesthetized SH rats.

**3** It was concluded that although captopril potentiated the vasodepressor action of Met-enkephalin in anaesthetized normotensive rats, potentiation of endogenous opioids does not appear to be involved in the hypotensive action of captopril in anaesthetized SH rats.

## Introduction

Angiotensin-converting enzyme (ACE) is a dipeptidyl-dipeptidase capable of cleaving the decapeptide angiotensin I to produce the potent, pressor octapeptide angiotensin II (Soffer, 1976). Captopril is an orally active and relatively specific inhibitor of ACE, as well as an effective antihypertensive agent in man and other animals (Heel, Brogden, Speight & Avery, 1980; Cushman, Cheung, Sabo & Ondetti, 1981).

While the hypotensive action of captopril is believed to be secondary to the blockade of ACE (Antonaccio, Rubin & Horovitz, 1980), the precise mechanism is unclear. Captopril has been shown to lower blood pressure of spontaneously hypertensive (SH) rats by a mechanism that is apparently independent of both the renal and vascular wall renin-angiotensin system (Hutchinson & Mendelsohn, 1980). The finding that both bradykinin (Dorer, Kahn, Lentz, Levine & Skeggs, 1974) and enkephalin (Erdos, Johnson & Boyden, 1978) are substrates for ACE raises the possibility that potentiation of either of these peptides by captopril could be involved in the acute hypotensive action of captopril in the anaesthetized SH rat. However, despite the finding that captopril increases the level of circulating kinins in experimental animals (McCaa, Hall & McCaa, 1978; Matthews & Johnston, 1979), bradykinin is not believed to play a major role in the hypotensive action of captopril in animals (see review Antonaccio *et al.*, 1980) or man (Millar & Johnston, 1979). The role of endogenous opioids in the hypotensive action of captopril has not yet been examined; however, the finding that captopril potentiates morphine-induced respiratory depression (Oktay, Onur, Mustafa & Turker, 1981) and analgesia (Ercan, Ilhan & Turker, 1980) suggests that ACE is involved in the metabolism of endogenous opiates and that captopril treatment may result in the accumulation of endogenous opioids.

Intravenous administration of the opioid peptide, methionine-enkephalin (Met-enkephalin) to the anaesthetized cat (Moore & Dowling, 1980) and rat (Wei, Lee & Chang, 1980) results in a rapid, transient fall in blood pressure. Given that the degradation of systemic enkephalin is believed to be by the action of a dipeptidyl dipeptidase associated with vascular endothelial tissue (Erdos *et al.*, 1978; Benuck & Marks, 1979), we have examined whether or not captopril can modify the acute vasodepressor action of exogenous Met-enkephalin in the anaesthetized rat. Furthermore, we have investigated, with the use of the opiate receptor antagonist, naloxone, whether or not potentiation of endogenous opioids is involved in the hypotensive action of captopril in the anaesthetized SH rat.

## Methods

### *Anaesthetized rat bioassay*

Male Wistar Kyoto rats (250–350 g) were anaesthetized with Inactin (sodium salt of 5-ethyl-5-(1[1]-methylpropyl)-2-thiobarbituric acid) (100 mg kg$^{-1}$, i.p.), an anaesthetic without any hypotensive action (Munoz-Ramirez, Khosla, Bumpus & Khairallah, 1978). Tracheostomy was performed and cannulae placed in the left jugular vein for drug injections and the left common carotid for the continuous recording of mean blood pressure on a Grass polygraph (Model 7C) via p23 dB Statham pressure transducers. Animals respired spontaneously; a moist oxygen supplement was supplied and body temperature was maintained at 36.5°C with an electric blanket.

### *Drug interactions*

Experiment 1: one hour after surgery, intravenous (i.v.) bolus injections of Met-enkephalin (Bachem) were given (10, 20, 40, and 80 μg kg$^{-1}$ each in 0.1 ml) in a randomized manner at 10 min intervals. Each animal then received either captopril (2 mg kg$^{-1}$, i.v.) or saline (0.1 ml, i.v.) and the doses of Met-enkephalin were repeated. In a further group of rats, the effect of naloxone (1.5 mg kg$^{-1}$, i.v.) on the response to a 40 μg kg$^{-1}$ dose of Met-enkephalin was studied over a 3 h period.

Experiment 2: The protocol of Experiment 1 was followed except that nitroprusside (Roche) was given in 3 doses (2.5, 5 and 10 μg kg$^{-1}$) instead of the 4 doses of Met-enkephalin.

Experiment 3: spontaneously hypertensive (SH) rats of the Okamoto strain (250–300 g) were prepared as above and 1 h after surgery they received either naloxone (1.5 mg kg$^{-1}$, i.v.) or vehicle (0.1 ml of 0.9% saline, i.v.). After a further 30 min each rat received a bolus injection of captopril (2 mg kg$^{-1}$, i.v.). Blood pressure was monitored continuously until 3 h after captopril had been given.

### *Statistics*

Two way analysis of variance (Armitage, 1977) was used to test for the significance of differences in responses to Met-enkephalin during the treatment periods and also to assess the effect of naloxone on the hypotensive action of captopril in SHR. Student's paired *t* test was also used to assess the significance of differences in responses to Met-enkephalin during the treatment periods.

## Results

Experiment 1: intravenous Met-enkephalin caused a dose-dependent, transient fall in blood pressure in anaesthetized rats. Expressed as a percentage fall in blood pressure, the response ranged from 8% at its lowest dose of 10 μg kg$^{-1}$ to 26% at 80 μg kg$^{-1}$. The vasodepressor response to each dose of Met-enkephalin following captopril was significantly greater than the corresponding pretreatment value ($F_{1,32} = 14.2$, $P < 0.05$), the results being shown in Table 1.

However, the vasodepressor responses to intravenous Met-enkephalin following the saline injection were not significantly different from the corresponding controls ($F_{1,24} = 1.1$, $P > 0.05$) (Table 1). Naloxone antagonized Met-enkephalin, the responses being about 80% reduced 3 h after naloxone was given.

Experiment 2: captopril in an identical regime to Experiment 1 failed to modify significantly the dose-dependent, transient hypotensive effect of nitroprusside in the anaesthetized rat whether the values were expressed as absolute or as percentage falls in blood pressure ($F_{1,18} = 0.1$, $P > 0.05$). The fall in blood pressure ranged from 25 mmHg at 2.5 μg kg$^{-1}$ to 50 mmHg at 10 μg kg$^{-1}$ nitroprusside.

Experiment 3: captopril (2 mg kg$^{-1}$, i.v.) caused a gradually developing fall in blood pressure, over 3 h, in anaesthetized SH rats ($n = 8$) pretreated with vehi-

**Table 1** Effects of captopril, naloxone or saline on vasodepressor responses to intravenous methionine-enkephalin (Met-enkephalin) in anaesthetized rats following the treatment regimes outlined in Experiment 1

| *Dose of Met-enkephalin* (μg kg$^{-1}$) | *Captopril* (2 mg kg$^{-1}$) | *Vehicle* (0.1 ml of 0.9% saline) | *Naloxone* (1.5 mg kg$^{-1}$, i.v.) |
|---|---|---|---|
| 10 | 273%* | 111% | — |
| 20 | 209 * | 102 | — |
| 40 | 169 * | 85 | 18*† |
| 80 | 155 * | 91 | — |

Results are expressed as a % of controls with 5–7 rats in each experiment.
*$P < 0.01$ when compared to control values as assessed by paired *t* test.
†Responses measured 3 h after naloxone treatment.

cle ($190 \pm 3$ to $172 \pm 3$ mmHg, $P < 0.005$) which was not significantly different ($F_{1,16} = 0.1$, $P > 0.05$) from that in SH rats pretreated with 1.5 mg kg$^{-1}$ naloxone ($193 \pm 4$ to $171 \pm 6$ mmHg).

## Discussion

In the present study captopril significantly potentiated the vasodepressor action of Met-enkephalin in anaesthetized rats. The altered responses to Met-enkephalin following captopril were not due to changes either in tissue sensitivity or in experimental protocol as captopril failed to alter the vasodepressor responses to the non-specific vasodilator, nitroprusside.

Since Met-enkephalin is a substrate for dipeptidyl dipeptidase (Erdos *et al.*, 1978), captopril may potentiate the action of circulating Met-enkephalin by the blockade of this enzyme. Captopril induces respiratory depression (Oktay *et al.*, 1981) and analgesia (Ercan *et al.*, 1980) in mice, effects which could occur by potentiation of endogenous opioids. The facts that Met-enkephalin is vasodepressor in the anaesthetized rat (Wei *et al.*, 1980) and that its action is augmented by captopril suggest that potentiation of endogenous opioids by captopril may contribute to its own hypotensive action in anaesthetized SH rats. However naloxone, at a dose known to antagonize powerfully the vasodepressor action of Met-enkephalin (Table 1), failed to alter significantly the hypertensive action of captopril in anaesthetized SH rats. Thus, it seems unlikely that potentiation of endogenous opioids contributes to the hypotensive action of captopril in these animals.

While captopril is a potent inhibitor of ACE and Met-enkephalin is a substrate for purified ACE, it is not known whether the effects of captopril on the actions of Met-enkephalin seen in this study were due to the inhibition of ACE and/or some other 'enkephalinase'. There is some evidence to suggest that, in some tissues, there exists an 'enkephalinase' that is distinct from ACE (Swerts, Perdrisot, Patey, De La Baume & Schwartz, 1979).

While intravenous Met-enkephalin decreases blood pressure in the anaesthetized rat (Wei *et al.*, 1980) and cat (Moore & Dowling, 1980) in the conscious rat, Met-enkephalin causes an increase in blood pressure (Simon, Schaz, Ganten, Stock, Schlor & Ganten, 1978). Thus, potentiation of this opioid by captopril is unlikely to be involved in the hypotensive action of captopril in the conscious SH rat. Nevertheless similar studies to the present ones are warranted in conscious animals.

This work was supported by the National Heart Foundation of Australia. We thank Dr G.J. Dusting for reviewing the manuscript.

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(*Received October 29, 1982.*
*Revised June 20, 1983.*)