MODIFICATION OF IN VITRO AND IN VIVO EFFECTS OF LEU- AND MET-ENKEPHALINS BY CAPTOPRIL

G. Ya. Shvarts and I. F. Faermark

UDC 615.357:577.175.852.042.2].015.4:612.884-06:]612.822.1:[547.95:547.943

KEY WORDS: captopril; Leu- and Met-enkephalins; analgesic effect.

The writers showed previously that the thiol kinase inhibitors (unithiol, D-penicillamine, cysteine [3]), and also captopril, depending on the dose used, may weaken or intensify pain responses induced in mice by various kinds of nociceptive stimulation. Pharmacological analysis showed that intensification of pain by the substances mentioned above is associated with delayed inactivation of kinins formed in response to nociceptive stimulation, whereas their analgesic effect is due to strengthening of activity of the endogenous antinociceptive system, for it was abolished by the opiate antagonist naloxone [4].

In the investigation described below the action of captopril was studied on the effects of Leu- and Met-enkephalins (LE and ME, respectively) in vitro and in vivo.

EXPERIMENTAL METHOD

The effect of captopril on LE and ME reception was studied in experiments on isolated segments of the ileum from guinea pigs of both sexes weighing 300-400 g, during their electrical stimulation by square pulses (T stimulator, from Hugo Sachs Elektronik, West Germany; parameters of stimulation 0.1 Hz, 2-4 msec, 20-60 V). Contractions of the ileum were recorded under isometric conditions on a Unirecord automatic writer (Ugo Basile, Italy). Captopril, LE, and ME (concentration range from 10^{-9} to 10^{-4} g/ml), dissolved in Tyrode solution (37°C), through which a gas mixture of 95% O_2 + 5% CO_2 was bubbled, were added directly to the receptacle containing the organ.

The action of captopril on the analgesic effect of LE and ME was studied in male mice weighing 20-22 g by the hot plate method [4]. Captopril (25 mg/kg) and naloxone (2.5 mg/kg) were injected subcutaneously 30 min before nociceptive stimulation, and LE and ME were injected intraperitoneally in a dose of 5 mg/kg. The effect of the substances on pain responses was assessed by measuring the change in the threshold of pain sensitivity (TPS) 3, 5, 15, 30, 60, and 120 min after injection.

The action of each dose of the substances was studied on 8-10 animals. Mice of the control groups received isotonic NaCl solution in the same volume and injected by the same method.

The experimental results were subjected to statistical analysis by Student's t test.

The captopril used in the experiments was obtained from Squibb (USA), the LE and ME from Serva (West Germany), and the naloxone (Narcan) from Du Pont (USA).

EXPERIMENTAL RESULTS

LE and ME caused a dose-dependent decrease in the amplitude of contractions of the guinea pig ileum induced by electrical stimulation (Table 1). When Tyrode solution containing captopril in the threshold concentration in which it caused minimal inhibition of contractions of the ileum (10^{-6} g/ml) was used, the action of LE and ME was significantly potentiated and prolonged (Fig. 1; Table 1). Whereas in the control LE, in a concentration of 10^{-7} g/ml, reduced the amplitude of contractions of the ileum to electrical stimulation by 38 ± 4.6%, in the presence of captopril (10^{-6} g/ml) the effect of LE was increased to 79 ± 18.8% ($p < 0.05$). Similar results were obtained also in the experiments with ME (Table 1).

Laboratory of Pharmacology, S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. D. Mashkovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 6, pp. 692-694, June, 1987. Original article submitted November 25, 1985.

0007-4888/87/0006-0785$12.50

TABLE 1. Effect of Captopril (10^{-6} g/ml) on Strength and Duration of Effects of LE and ME on Isolated Guinea Pig Ileum during Its Electrical Stimulation

Concentration of enkephalins, g/ml	Inhibition of contractions, % of original (M ± m)				Duration of inhibition (M ± m), min			
	LE	LE + captopril	ME	ME + captopril	LE	LE + captopril	ME	ME + captopril
10^{-7}	38±4,7	79±18,8**	33±6,3	67±10,5**	5±0,8	12±1,3***	6±2,1	9±1,7*
$2 \cdot 10^{-7}$	40±8,4	82±12,6**	43±14,7	78±10,0**	6±0,7	11±1,2***	9±2,5	11±0,8*
$5 \cdot 10^{-7}$	61±2,1	87±8,4**	55±9,7	95±4,2**	11±2,1	17±2,3**	10±3,0	16±1,7**
10^{-6}	81±2,8	93±8,1**	73±10,5	97±2,1**	12±3,8	22±4,2**	12±3,4	21±1,3**
$2 \cdot 10^{-6}$	85±4,5	98±2,1**	78±9,3	100**	13±3,7	40±10,9**	15±3,1	28±1,7***
$5 \cdot 10^{-6}$	95±2,6	100*	93±6,3	100*	16±4,2	57±13,0**	20±3,4	45±4,7***

Legend. *p > 0.05, **p < 0.05, ***p < 0.001.

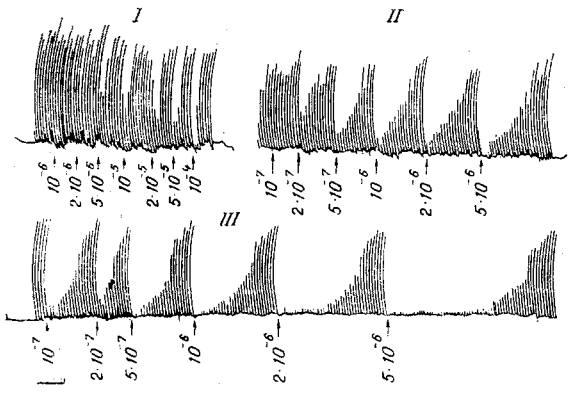

Fig. 1

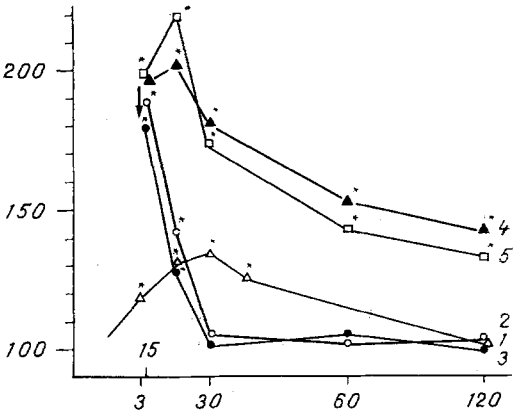

Fig. 2

Fig. 1. Effect of captopril (I), LE (II), and captopril (10^{-6} g/ml) + LE (III) on contractions of isolated segment of guinea pig ileum during its electrical stimulation. Arrows indicate injection of drugs, numbers show concentration (in g/ml). Time marker 60 sec.

Fig. 2. Effect of captopril on analgesic effect of LE and ME. Abscissa, time (in min); ordinate, TPS (in % of control). 1) Captopril (25 mg/kg, subcutaneously); 2) LE (5 mg/kg, intraperitoneally); 3) ME (5 mg/kg intraperitoneally); 4) captopril + LE; 5) captopril + ME. *p < 0.05 compared with control.

Meanwhile the duration of action of LE and ME also was increased. In the control, recovery of the original amplitude of contraction after treatment with LE and ME in a concentration of $5 \cdot 10^{-6}$ g/ml took place after 16 ± 4.2 and 20 ± 3.4 min, respectively, whereas in the presence of captopril (10^{-6} g/ml) the time was increased to 57 ± 13.0 and 45 ± 4.7 min (Fig. 1; Table 1).

In the experiments in vitro captopril, in a concentration not significantly changing the character of contractions of the guinea pig ileum to electrical stimulation, thus potentiated and prolonged the inhibitory effects of LE and ME.

In mice, using a model of painful thermal stimulation, LE and ME, injected intraperitoneally in a dose of 5 mg/kg, more than doubled the level of TPS. Their effect reached a maximum by 3-15 min after injection, after which it decreased rapidly (Fig. 2). After preliminary injection of captopril the analgesic effect of both enkephalins was strengthened and prolonged, and this effect lasted during 2 h of observation and was prevented by naloxone (Fig. 2).

These experiments thus show that captopril, acting both on isolated organs and on the body as a whole, potentiates and prolongs the effect of LE and ME. Abolition of the enkephalin-potentiating action of captopril by naloxone is evidence that the enkephalins participate in the realization of the analgesic action of this drug.

When these results are analyzed it must be recalled that captopril, when administered by different methods, is not found in the brain, because it does not pass through the blood-brain barrier [1, 2, 6].

Consequently, direct interaction between captopril and enkephalin-metabolizing enzymes contained in the brain (enkephalinase A, kininase II, etc.), evidently does not take place. More recently, however, it has been reported that activity of brain kininase II and "enkephalinase" is inhibited during systemic administration of captopril [5], which indicates that these enzymes may be inhibited indirectly in the central nervous system by captopril. The possibility cannot be ruled out that the presence of a thiol group in its molecule plays a role in the mechanism of action of captopril. In fact, the kininase II inhibitor enalapril (MK-421), which does not contain a thiol group, has no analgesic action and does not change the effect of morphine in rats in doses causing virtually complete inhibition of this enzyme [6].

LITERATURE CITED

1. V. M. Bulaev, Progress in Science and Technology, Series: Pharmacology, Chemotherapeutic Agents [in Russian], Vol. 13, Moscow (1982), pp. 101-184.
2. A. V. Val'dman and O. S. Medvedev, Vest. Akad. Med. Nauk SSSR, No. 5, 14 (1982).
3. G. Ya. Shvarts, Farmakol. Toksikol., No. 5, 606 (1981).
4. P. A. J. Janssen and A. Jageneau, J. Pharm. Pharmacol., 9, 381 (1957).
5. K. J. Kripalani, D. N. McKinstrey, and S. M. Singhvi, J. Clin. Pharmacol., 27, 636 (1980).
6. P. Mojaverion, B. N. Swanson, and R. K. Ferguson, Eur. J. Pharmacol., 98, 303 (1984).