EFFECT OF N-FORMYL-L-METHIONYL-L-LEUCYL-L-PHENYLALANINE AND ITS ANALOGUES ON BLOOD PRESSURE

HARUO SAITO, MOTOYA MATSUO, SHIGERU NAMINOHIRA, TAKESHI SAKAI,* HARUO KITAGAWA,** AND ARTHUR A. HIRATA***

Department of Toxicology, School of Pharmacy, Hokuriku University,* Ho-3, Kanagawa-machi, Kanazawa 920-11, Japan, Department of Drug Evaluation and Toxicological Sciences, Faculty of Pharmaceutical Sciences, Chiba University,** 1-33, Yayoi-cho, Chiba 260, Japan and Department of Microbiology, University of Kansas,*** Lawrence, Kansas 66045, U.S.A.

(Received October 19, 1982)

Effects of N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), the synthetic chemotactic peptide, and its analogues on blood pressure were investigated in the rabbit, rat, mouse, dog, cat and guinea pig. The administration of FMLP (5 μg/kg i.v.) induced the depressor effect in the rabbit but no effect was observed in the other animal species. The only peptide which showed a similar effect as FMLP was N-formyl-L-methionyl-L-methionyl-L-phenylalanine (FMMP) among the peptides relating to FMLP. Tachyphylaxis was observed in the hypotensive action induced by the two peptides.

Keywords — N-formyl-L-methionyl-L-leucyl-L-phenylalanine; blood pressure; species difference; tachyphylaxis

INTRODUCTION

Certain synthetic chemotactic peptides bind to their specific receptors on the cell membrane and induce chemotaxis, granule enzyme secretion (in the presence of cytochalasin B), aggregation and triggering of the respiratory burst of polymorphonuclear leukocytes (PMN).1) The most active synthetic tripeptide presently known is N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP).2) Although many studies on this peptide have been performed, its effect on the cardiovascular system has not been reported. In this study, we investigated the effect of FMLP and its analogues on blood pressure in various species of animals.

MATERIALS AND METHODS

Male ICR mice (30–40 g), male Sprague-Dawley rats (300–400 g), male Hartley guinea pigs (400–500 g), male New Zealand white rabbits (2–3 kg) and male crossbred cats and dogs (2–3 kg and 7–8 kg, respectively) were anesthetized with urethane (1.5–2.0 g/kg, s.c.) or sodium pentobarbital (35 mg/kg, i.v.). The carotid and femoral veins were cannulated. Blood pressure recordings were made via the carotid cannula using a pressure transducer (Nihon-Koden MPU-0.5) connected to a recorder (Tokai-Irika TI-102). The mean blood pressure was designated as the diastolic pressure plus 33% of value (mmHg) subtracting diastolic pressure from systolic pressure. Arachidonic acid was dissolved in 0.5 m Na2CO3 (10 mg/ml) and diluted further with 0.9% NaCl solution (1 mg/ml). Indomethacin was dissolved in 0.1N NaOH. Other drugs were dissolved in 0.9% NaCl solution and were administered from the femoral vein. The following drugs were used. N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, Vega Biochemical, Tucson, Arizona, U.S.A.), N-formyl-L-methionine (FM, Vega), N-formyl-L-phenylalanyl-L-methionine (FPM, Vega), N-formyl-L-methionyl-L-methionyl-L-phenylalanine (FMMP, Miles), N-formyl-L-methionyl-L-phenylalanine (FMP, Sigma), glycyl-L-phenylalanine (GP, Fluka), glycyl-
L-leucyl-L-tyrosine (GLT, Fluka), l-leucyl-glycyl-glycine (LGG, Fluka), l-leucine (Wako Pure Chemical), L-phenylalanine (Wako Pure Chemical), indomethacin (IM, Sumitomo), arachidonic acid (AA, Wako Pure Chemical).

RESULTS
The control blood pressure in rabbits was 77.8±2.8 mmHg (mean ± s.e. from 25 experiments). Following intravenous administration of FMLP (5 µg/kg) into rabbits, the blood pressure changed in a triphasic pattern. After FMLP was injected, it reduced the blood pressure to 48.2±3.8 mmHg (n = 6) in about 60 s, returned to 57.4±4.6 mmHg after about 120 s and reduced it to 41.4±2.0 mmHg again after about 260 s. This hypotensive effect was dose-dependent at dose levels of 0.5 to 5 µg/kg. The

**FIG. 1. Effect of FMLP on the Blood Pressure in Rabbit, Mouse, Rat, Guinea Pig, Cat and Dog**

△; FMLP (5 µg/kg i.v.). ○; norepinephrine (10 µg/kg i.v.). ●; acetylcholine (1 µg/kg i.v.).

**FIG. 2. Effect of FMLP-analogues (5 µg/kg i.v.) on the Blood Pressure in Rabbits**

○; norepinephrine (10 µg/kg i.v.). ●; acetylcholine (1 µg/kg i.v.).
Hypotensive Effect of FMLP

depression lasted for about 45 min and tachyphylaxis of the depression was observed after the return of the depression (Fig. 1). Following the intravenous administration of FMLP (5 μg/kg) into the mice, rats, guinea pigs, cats and dogs, it can be seen that the blood pressure did not change significantly (Fig. 1).

Effect of the analogues and other peptides on the blood pressure in rabbits were also examined. When FMMP (5 μg/kg i.v.) was injected, the blood pressure changed in a triphasic pattern. After FMMP was injected, it reduced the blood pressure to 47.0±12.1 mmHg (n=3) in about 90 s, returned to 61.0±6.7 mmHg after about 170 s and reduced it to 41.5±5.4 mmHg again after about 430 s. The depression lasted for approximately 45 min and tachyphylaxis of the depression was observed after the return of the depression (Fig. 2). No other drugs altered the blood pressure at dose levels of 5 and 50 μg/kg (Fig. 2, all data are not shown).

As shown in Fig. 3, the first phase in FMLP-induced hypotension was blocked by the pretreatment of IM (10 mg/kg s.c.). Similarly, the depressor effect of AA (0.5 mg/kg i.v.) was completely blocked by IM.

DISCUSSION

The present experiments demonstrate that FMLP injected intravenously reduces blood pressure in anesthetized rabbits. It was reported that FMLP exerted the most potent ability of chemotaxis and lysozyme secretion activities in rabbit PMN of all synthetic formylmethionyl peptides.2)

The depressor effect induced by FMLP may be due to the direct action to PMN, because FMMP, which has relatively high activity in chemotaxis and lysosomal enzyme secretion,2) decreased blood pressure in rabbits but FMP and FM with a low activity2) did not decrease, and chemotactic peptides released arachidonic acid from rabbit PMN and the intensity of the action was parallel to that of chemotactic ability.3)

On the other hand, it was reported that chemotactic and lysozyme secretion activities of peptides to PMN disappeared after their preincubation with PMN.4,5) The facts agree with our observation that tachyphylaxis appeared in the depressor effect of FMLP. Furthermore, our discovery that the depressor effect was recognized in rabbit but not in other animals used also agree with the reports that a specific receptor on the PMN membrane to formyl peptides was recognized only in rabbit6) and human.7) These two facts supported the above consideration.

In addition, the first phase in FMLP-induced hypotension and the depressor effect of AA were blocked by IM, the inhibitor of cyclooxygenase. It has been reported that FMLP released AA from rabbit PMN in vitro3) and it is widely known that some prostaglandins from AA metabolites in the cyclo-oxygenase system evoke a hypotensive effect. According to this fact, we suggested that the depressor effect of FMLP in rabbits may be due to the AA metabo-
lite(s) being released from PMN in the interaction between FMLP and a receptor on the membrane of PMN.

REFERENCES