6.47 (1H, d, $\underline{H}_{(4)}$>=<, J=3 cps), 7.15 (1H, d, $\underline{H}_{(3)}$>=<, J=3 cps), 9.25 (1H, s, -C$\underline{H}$O).

II was identified with an authentic sample by IR, NMR, TLC and MS.

Acknowledgement We thank Mr. Haruaki Mori for his assistance. We are also indebted to Dr. K. Sasaki, Chemical Institute, Faculty of Science, Nagoya University, for mass spectral measurements and Analytical Center of our University for NMR spectra and elemental analyses.

[Chem. Pharm. Bull. 21(5)1157—1160(1973)]

UDC 547.466.1.09 : 615.22.076.9

Cardiovascular Effects of Some Peptide Analogues consisting of Cystine and/or Tyrosine

Yoshikazu Yamatake, Hitoshi Kato, and Keijiro Takagi

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo[1)]

(Received November 30, 1972)

Compounds that competitively block the pharmacological effects of posterior pituitary hormones have been sought for a long time. Some peptides containing tyrosine and/or cystine were synthesized for this purpose, and examined to have a competitive inhibitory effect on the contraction induced by oxytocin in the rat uterus by Ishida, *et al.*[2–4)]

In the present study, we studied the effect of such synthetic peptides on the cardiovascular system as well as on the pressor action of vasopressin.

Experimental

Method——1. Blood Pressure and Heart Rate in Anesthetized Rats: Male rats (Donryu strain, 240—460 g) were anesthetized with a mixture of urethane (600 mg/kg) and α-chloralose (60 mg/kg) intraperitoneally. Arterial blood pressure was taken from the cannulated carotid artery on a polygraph (Nihon Kohden, RM-150) using a pressure transducer (Nihon Kohden, MP U-0.5). Heart rate was measured by an instantaneous ratemeter (Nihon Kohden, RT-2) triggered from the pulse pressure. Drug solutions were injected into the cannulated femoral vein in volumes of 0.2 ml.

2. Peripheral Blood Flow in Anesthetized Dogs: The effects of the peptides on peripheral vascular resistance were studied in the autoperfused dog hindquarter and coronary beds. Male mongrel dogs weighing about 10 kg were anesthetized with sodium pentobarbital, 35 mg/kg, intravenously, the left femoral artery was cannulated, and blood flow into the left hindquarter was recorded with an electromagnetic flowmeter (Nihon Kohden, MF-2). Similarly, coronary blood flow was measured from the left anterior descending coronary artery which was perfused with the blood derived from the left carotid artery in an anesthetized open-chest dog under artificial respiration. Peripheral vascular resistance was calculated as the ratio of pressure to flow. Drugs were injected intraarterially in volumes of 0.4 ml for ten seconds.

Materials——The synthetic peptides examined are as follows:

I: L-cystine diethylester (CyS–OEt)$_2$·2HCl
II: L-cystinyl-di-L-tyrosine ethylester (CyS–Tyr–OEt)$_2$·2HBr
III: L-cystinyl-di-L-tyrosyl-L-tyrosine ethylester (CyS–Tyr–Tyr–OEt)$_2$·2HBr
IV: di-carbobenzoxy-L-cystinyl-di-L-tyrosyl-L-tyrosine ethylester (Cbz–CyS–Tyr–Tyr–OEt)$_2$
V: L-tyrosyl-L-tyrosine ethylester Tyr–Tyr–OEt·HBr

1) Location: *Hongo 7-3-1, Bunkyo-ku, Tokyo.*
2) Y. Ishida, *Yakugaku Zasshi*, **81**, 1722 (1961).
3) Y. Ishida and K. Hara, *Chem. Pharm. Bull.* (Tokyo), **12**, 872 (1964).
4) Y. Ishida and M. Onishi, *Chem. Pharm. Bull.* (Tokyo), **14**, 748 (1966).

Peptides I, II and III were dissolved with saline. Peptide IV was dissolved with propyleneglycol-saline 9:2 and V with propyleneglycol–ethanol–saline 2:1:5. Glyceryl trinitrate (Nihon Kayaku), papaverine hydrochloride (Iwaki Seiyaku) and Pitressin (Parke Davis) as a vasopressin preparation were also used.

Result

1. Effects of the Peptides on Blood Pressure and Heart Rate in Rats

Peptides I, II, and III were injected at a dose range of 0.5 to 16 mg/kg. Administration of these peptides usually resulted in a fall in blood pressure accompanied by a decrease in heart rate. Typical records of I and II are shown in Fig. 1. The changes in blood pressure observed are summarized in Fig. 2, where the magnitude (mmHg) of the maximal depressor responses to these compounds is shown at each dose. Effects of the peptides IV and V were evaluated by deduction of the effect of solvents. As seen in Fig. 2, changes of blood pressure and heart rate after peptides I, II, and III were almost dose-related, but those of IV and V were not. The depressor potency of the peptides in the molar basis was as following order; III>II>I>IV=V.

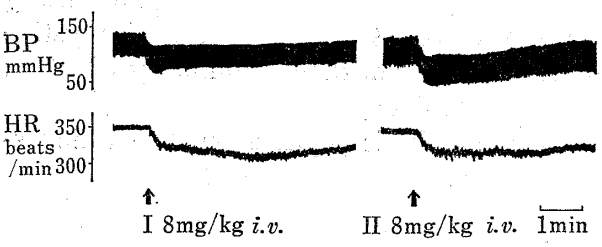

Fig. 1. Effect of the peptides I and II on Carotid Arterial Blood Pressure(BP) and Heart Rate (HR) in Anesthetized Rats

Responses to peptide I and II (8 mg/kg *i. v.*)

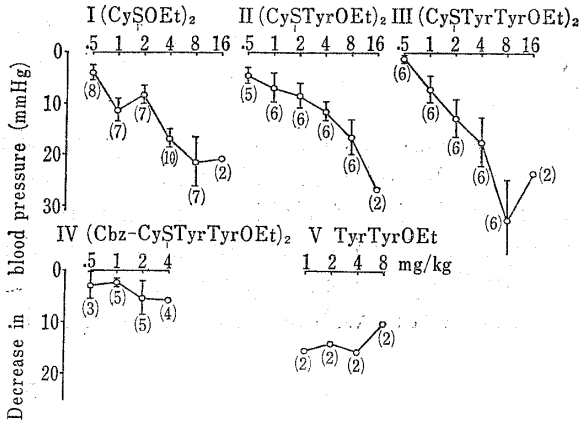

Fig. 2. Dose-Response Curves for the Synthetic Five Peptides (I, II, III, IV and V) in the Fall of Carotid Arterial Blood Pressure in Anesthetized Rats

The vertical bars represent the standard errors of the means. Figures in the paranthesis refer to number of animals in each experiment.

2. Antagonistic Activity of the Peptides to Vasopressin

Antagonistic activity to vasopressin was determined by the changes of blood pressure in anesthetized rats except for peptide IV. Maximal values of the pressor effects of vasopressin, 0.04 units/kg *i.v.*, were compared before and 5—8 minutes after the administration of 16 mg/kg *i.v.* of each synthetic peptide. No tachyphylaxis to vasopressin was observed in this dose injected at a several minutes interval. Summary of the results obtained is shown in Table I. As seen in the table, no substantial difference was observed between pre- and post-responses to vasopressin.

3. Effects of the Peptides on Peripheral Blood Flow in Dogs

Six dogs were used in hindquarter perfusion and one in coronary perfusion experiments. The effects of the peptides on each peripheral resistance except for IV were compared with those of papaverine and glyceryl trinitrate. In hindquarter vascular beds a close-arterial injection of peptide I caused a significant transient increase in blood flow, and vasodilatory activities of 1.5 and 3 mg *i.a.* of the compound were comparable to papaverine, 200 μg *i.a.*,

Table I. Effect of the Synthetic Petpides I, II, III, and IV on Pressor Responses to Pitressin (0.04 units /kg *i.v.*)

Treatment	Dose mg/kg *i.v.*	Number of animals	Rise in blood pressure (mmHg±S.E.)	
			Pitressin before treatment	Pitressin after treatment
Peptide I	16	6	35±9	33±9
II	16	5	25±5	24±6
III	16	5	24±5	19±5
IV	16	2	32	30

and glyceryl trinitrate, 4 μg *i.a.* (Fig. 3). Peptide V produced more prolonged vasodilation. The potency of these peptides for reduction of vascular resistance in the hindquarter was as following order in the molar basis; I>III>V>II.

In a coronary perfusion experiment, peptides I and II (3 mg, *i.a.*) were examined. Peptide I produced the same degree of vasodilation as in the hindquarter vascular beds and II (3 mg, *i.a.*), which had little effect on hindquarter blood flow, produced an apparent increase in the coronary blood flow (Fig. 4).

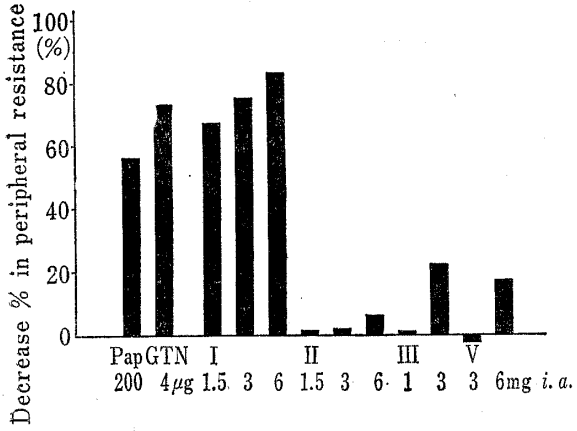

Fig. 3. Effect of the Peptides (I, II, III and V) on the Femoral Arterial Blood Flow in Anesthetized Dog

Papaverine (pap, 200 μg) and glyceryl trinitrate (GTN, 4 μg) were used for reference. All drugs were injected into the femoral artery. Data are means of six experiments.

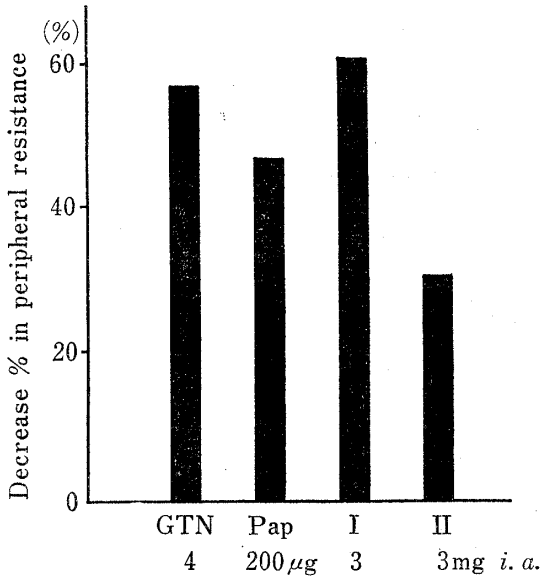

Fig. 4. Effect of the Peptides (I and II) on the Coronary Arterial Blood Flow in Anesthetized Dog

Papaverine (Pap, 200 μg) and glyceryl trinitrate (GTN, 4 μg) were used for reference. All drugs were injected into the left anterior descending coronary artery.

Discussion

Most peptides used in the present study contain cysteine-cysteine bond, since it is known that the integrity of this bond is essential for the activities of vasopressin and oxytocin.[5] In most compounds, tyrosine ethylester molecules are connected to this bond, which is expected to possess an antagonistic activity. Carbobenzoxyl groups which protected N-terminals

5) L.S. Goodman and A. Gilman (ed.), "The Pharmacological Basis of Therapeutics," The Macmillan Company, New York, 1970.

in the synthetic process were remained in peptide IV. From the results of this study (Fig. 3), however, no peptides employed modified the pressor effect of vasopressin even in considerably large doses up to 16 mg/kg *i.v.*, although they reduced blood pressure and heart rate in rats. Consequently, it seems reasonable to conclude that they would possess no antagonistic activity against vasopressin on rat blood pressure. These results are in contrast to those of the antagonistic action to oxytocin in the isolated rat uterus.[3,4] This discrepancy may be due to the organ difference or difference of the active sites of vasopressin from those of oxytocin.

Most of the peptides produced, more or less, vasodilation in dog hindquarter and coronary vascular beds, although the coronary perfusion was undertaken only in one animal due to the deficiency of the peptides available. It is indicated that in both the blood pressure lowering and vasodilatory effects, the potencies of the peptides examined did not correlate with the length of amino acid sequence.

Acknowledgement We are grateful to Prof. Y. Ishida and Dr. H. Moritoki, University of Tokushima, for the supply of the peptide analogues used in this study.

[Chem. Pharm. Bull. 21(5)1160—1163(1973)] UDC 547.291.04 : 547.468.04

Reaction of Alkyl Dialkoxyphosphinyl Formate with Strong Base

Shunsaku Shiotani and Tadashi Kometani

Toyama Technical College[1]

(Received November 30, 1972)

In 1966, Shahak[2] reported that ketones were carbalkoxylated in the α-position with ethyl diethoxyphosphinyl formate (EDEPF) in the presence of sodium hydride, *via* an intermediate $[R\cdot CO\cdot CR'\cdot CO\cdot P(=O)(OEt)_2]^-Na^+$, in good yield.

We attempted to apply this method to the preparation of ethyl 2-oxo-5-phenylcyclohexanecarboxylate from 4-phenylcyclohexanone (I). However, the sole product isolated from the reaction mixture was 1-diethoxyphosphinyl-4-phenylcyclohexanol (III), mp 110—111°. Therefore, we reexamined the reaction of cyclohexanone (II) with EDEPF according to the Shahak's procedure, and could not obtain the β-ketoester but 1-diethoxyphosphinylcyclohexanol (IV).[3]

On the other hand, it had been reported that EDEPF was decomposed by refluxing with aqueous sodium hydroxide solution into carbon dioxide and phosphorous acid, accompanying formation of phosphonoformic acid in low yield.[4] This fact implies that in a protic solvent hydroxide ion attacks at both the carbonyl carbon and the phosphorus atom of EDEPF. Thus, we examined the behavior of EDEPF, methyl diethoxyphosphinyl formate (MDEPF), ethyl diemthoxyphosphinyl formate (EDMPF) and methyl dimethoxyphosphinyl formate (MDMPF) with several strong bases, such as hydride, amide, ethoxide and methoxide anion, in benzene, in order to search any clue to resolve the above discord and to find the optimum condition for the carbalkoxylation of ketones with alkyl dialkoxyphosphinyl formate. The results are illustrated in Table I.

---

1) Location: *Hongo, Toyama.*
2) I. Shahak, *Tetrahedron Letters*, **1966**, 2201.
3) V.S. Abramov, *Zhur. Obshchei Khim.*, **22**, 647 (1952) [*C. A.*, **47**, 5351 (1953)].
4) *a*) P. Nylen, *Ber.*, **57**, 1023 (1924); *b*) S. Warren and M.R. Williams, *J. Chem. Soc.* (*B*), **1971**, 618.