and XII). The sample was distilled under reduced pressure (b.$p_{2\sim3}$ 72~78°) to afford 2.17 g. of a mixture. NMR spectra showed that this was a 1:1 mixture of XI and XII (Fig. 2a).

Preparation of IV from XIII——XIII was prepared from XI essentially according to Koegle, *et al.*[3] XIII·HCl (0.35 g.) was refluxed with HCOOH (30 ml.) for 3 hr. and cooled. Concentration of the mixture *in vacuo* gave a residue; this was dissolved in conc. HCl (3 ml.) and to the solution was added an equal volume of EtOH to afford 0.12 g. (34%) of IV; m.p. 230~233°. UV spectrum of this sample was identical with that of a sample of 3-deazaadenine, prepared by Koegle's method.[3] IV was converted into the picrate whose IR spectrum was also identical with that of the authentic sample, prepared by the unambiguous synthesis.[3] *Anal.* Calcd. for $C_6H_8N_4Cl_2$ (dihydrochloride of IV): C, 34.80; H, 3.89; N, 27.06. Found: C, 34.83; H, 4.00; N, 26.65.

The authors wish to thank Dr. Ken'ichi Takeda, Director of the Shionogi Research Laboratories, for the determination of NMR spectra. They also wish to thank Mrs. Toyoko Tohma and Miss A. Maeda for the elementary analysis.

Summary

The evidences that the reported 6-chloro-3,4-diaminopyridine was actually 2-chloro-3,4-diaminopyridine (X) were presented. By employing X 4-amino-1*H*-imidazo[4,5-*c*]-pyridine (IV) was prepared *via* 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (III). An alternative synthetic method leading to IV was devised using 1*H*-imidazo[4,5-*c*]pyridine 5-oxide (II) as a key intermediate as follows. Oxidation of 1*H*-imidazo[4,5-*c*]pyridine (I) with hydrogen peroxide in acetic acid afforded the cerresponding 5-oxide (II); treatment of II with boiling phosphoryl chloride gave rise to III. 3-Nitro-2,4-dichloropyridine, useful intermediate for the synthesis of IV (*via* 3-nitro-2,4-diaminopyridine→2,3,4-triaminopyridine) was prepared by N-oxide-phosphoryl chloride reaction from 3-nitro-4-chloropyridine 1-oxide. Comparison of the several routes leading to IV has been made from a standpoint of the over-all yield.

(Received March 27, 1964)

[Chem. Pharm. Bull. 12 (8) 872 ~ 877]

UDC 615.766-015

123. Yukio Ishida and Kazuko Hara: Studies on Inhibitory Actions of Synthetic Peptides on the Effects of Oxytocin and Vasopressin.

(*Pharmaceutical Faculty, University of Tokushima*[*1])

On the way of our studies with drug receptors of acetylcholine, barium chloride and oxytocin on the rat uterus, we found that the action of oxytocin was inhibited competitively by hydrogen ion and simple phenolic compounds. These results suggested that one of the active centres of oxytocin was tyrosine molecule in the peptide.[1~4]

In this report, many peptides containing tyrosine were synthesized for the purpose of obtaining more potent antagonistic substances against oxytocin. They showed more or less competitive inhibition to the contraction of rat uterus by oxytocin. Some peptides that did not contain tyrosine, on the other hand, exhibited no inhibitory action.

*1 Sho-machi Tokushima (石田行雄, 原 和子).

1) K. Takagi, Y. Ishida, H. Moritoki, K. Hara: Yakugaku Zasshi, **81**, 1708 (1961).
2) Y. Ishida, H. Moritoki, K. Hara: *Ibid.*, **81**, 1713 (1961).
3) Y. Ishida: *Ibid.*, **81**, 1717 (1961).
4) *Idem*: *Ibid.*, **81**, 1722 (1961).

Because ethyl carbobenzoxytyrosyl-tyrosinate was the most effective one among these peptides, its effects on the dose-response curves of acetylcholine, barium chloride and oxytocin were tested on the isolated rat uterus.

In addition to the above experiments, antagonistic actions of these peptides to the depressor effect of oxytocin on avian blood pressure and to the pressor effect of vasopressin on rabbits and dogs were tested.

Method and Materials

(1) On the Rat Uterus

Following the earlier report,[1] the uterus of ovary-ectomized rats was used.

(2) Peptides Used as Materials

Carbobenzoxy peptides were prepared by azide method or DCC (dicyclohexylcarbodiimide) method.

Ethyl carbobenzoxyglycyl-glycinate=Cbz-Gly-Gly (COOEt) m.p. 82°
Ethyl carbobenzoxy-L-leucyl-glycinate=Cbz-Leu-Gly (COOEt) m.p. 97~100°
Di-carbobenzoxy-L-cystinyl-di-L-tyrosinamide[5]=(Cbz-Cy-Tyr ($CONH_2$))$_2$ m.p. 226°
S-
Di-carbobenzoxy-L-cystinyl-di-L-tyrosine ethyl ester=(Cbz-Cy-Tyr (COOEt)$_2$ m.p. 174°
S-
Ethyl L-tyrosinate=Tyr (COOEt) m.p. 108°
Ethyl carbobenzoxyglycyl-L-tyrosinate=Cbz-Gly-Tyr (COOEt) m.p. 124°
Ethyl carbobenzoxy-L-alanyl-L-tyrosinate=Cbz-Ala-Tyr (COOEt) m.p. 142°
Ethyl carbobenzoxy-L-tyrosyl-L-tyrosinate=Cbz-Tyr-Tyr (COOEt) m.p. 160°

Being insoluble in H_2O, these peptides were dissolved in small amount of propylene glycol (1:10), and then diluted with physiological saline solution.

Experimental Results

1) Screening Test of Inhibitory Activities of Synthetic Peptides to Oxytocin on the Isolated Rat Uterus

The inhibitory activities of these peptides and *p*-nitrophenol in the concentration of 5×10^{-5} g./ml. were estimated to the contraction produced by oxytocin 2×10^{-4} u./ml. Some of peptides which showed the inhibition lower than 10 per cent (that is, the contraction heights were more than 90 per cent) were estimated once more with 10^{-4} g./ml. of them.

Table I. Screening Test of Inhibitory Activities of Carbobenzoxypeptides to Oxytocin

Antagonists	with 5×10^{-5} g./ml.		with 10^{-4} g./ml.
	Contraction	Inhibition	Contraction
Cbz-Gly-Gly (COOEt)	95.7	4.3	95.6
Cbz-Leu-Gly (COOEt)	99.4	0.6	103.5
(Cbz-Cy-Tyr ($CONH_2$))$_2$ S-	98.3	1.7	99.6
(Cbz-Cy-Tyr (COOEt))$_2$ S-	80.4	19.6	—
Tyr (COOEt)	75.3	24.7	—
Cbz-Gly-Tyr (COOEt)	52.2	47.8	—
Cbz-Ala-Tyr (COOEt)	45.9	54.1	—
Cbz-Tyr-Tyr (COOEt)	11.5	88.5	—
p-Nitrophenol	53.5	46.5	—

Standard contraction by oxytocin 2×10^{-4} u./ml. is 100 per cent. Each values are percentages of contraction by oxytocin 2×10^{-4} u./ml. with antagonists.

5) E. Wintersberger, H. Tuppy, E. Stoklaska : Monatsh. Chem., **91**, 578 (1960).

Ethyl carbobenzoxyglycyl-glycinate and ethyl carbobenzoxyleucylglycinate which do not contain tyrosine molecule did not show any inhibitory activities, however peptides containing tyrosine had more or less activities. Most of them were about as potent as *p*-nitrophenol, but only ethyl carbobenzoxytyrosyl-tyrosinate was much stronger than *p*-nitrophenol.

2) Relative Inhibitory Activities on the Contraction of Uterus by Oxytocin between Ethyl Carbobenzoxytyrosyl-tyrosinate and *p*-Nitrophenol

From the result of the above screening test, we tried to estimate effective dose (ED_{50}) of the inhibitory activities of ethyl carbobenzoxytyrosyl-tyrosinate comparing with *p*-nitrophenol. Ethyl tyrosinate was also tested. Ethyl carbobenzoxytyrosyl-tyrosinate was 30.4 times as potent as *p*-nitrophenol on the molar basis.

Table II. Inhibitory Activities against Oxytocin on Uterus Contraction (Molar Basis)

Compd.	Concn. (*M*)	Contraction[a] (%)	ED_{50} (*M*)	Potency ratio[b]
p-Nitrophenol	9.30×10^{-5}	90.6	1.58×10^{-4}	1.00
	1.86×10^{-4}	36.4		
Cbz-Tyr-Tyr (COOEt)	1.97×10^{-6}	82.5	5.20×10^{-6}	30.4
	4.94×10^{-6}	53.5		
	9.87×10^{-6}	27.9		
Tyr (COOEt)	1.45×10^{-3}	51.9	1.45×10^{-3}	0.11
	2.86×10^{-3}	8.41		

a) Contractions of oxytocin 2×10^{-4} u./ml. with antagonists.
b) ED_{50} of *p*-nitrophenol/ED_{50} of other compounds.

3) Antagonism of Ethyl Carbobenzoxytyrosyl-tyrosinate against ACh, Barium Chloride and Oxytocin

Against ACh and barium chloride: After the dose-response curve of ACh had been estimated on isolated rat uterus, the dose-response curves were obtained in the presence of ethyl carbobenzoxytyrosyl-tyrosinate 5×10^{-5} g./ml. and 10^{-4} g./ml. Against barium chloride it was used in 10^{-5} g./ml. and 2×10^{-5} g./ml. (Fig. 1 and Fig. 2). Each datum (%) represents a mean of 4 experiments.

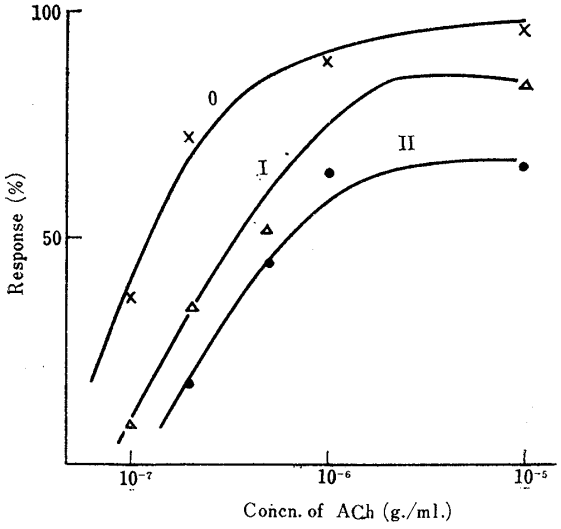

Fig. 1. Movement of Dose-Response Curves of ACh
Concn. of Cbz-Tyr-Tyr (COOEt): 0, ACh alone; I, 5×10^{-5} g./ml.; II, 10^{-4} g./ml.

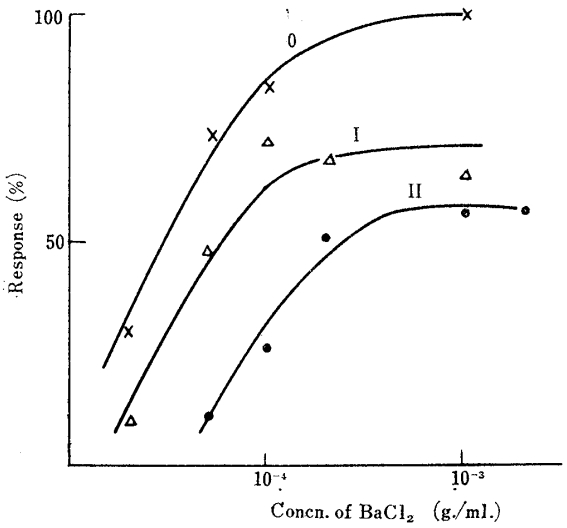

Fig. 2. Movement of Dose-Response Curves of Barium Chloride
Concn. of Cbz-Tyr-Tyr (COOEt): 0, $BaCl_2$ alone; I, 10^{-5} g./ml.; II, 2×10^{-5} g./ml.

As both maximum contractions were inhibited, these antagonism seemed to be non-competitive as in the case of *p*-nitrophenol.

Against oxytocin : The contraction of oxytocin was inhibited competitively by ethyl carbobenzoxytyrosyl-tyrosinate at 2×10^{-6} g./ml., 4×10^{-6} g./ml., and 8×10^{-6} g./ml., respectively (Fig. 3a and Fig. 3b). Each datum (%) represents a mean of 4 experiments.

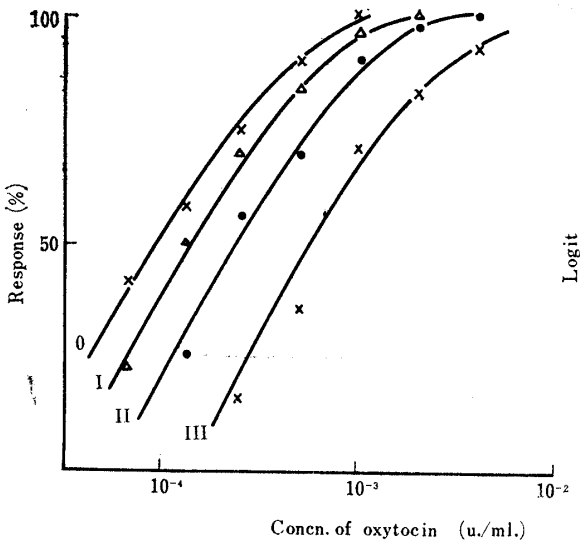

Fig. 3a. Movement of Dose-Response Curves of Oxytocin
Concn. of Cbz-Tyr-Tyr (COOEt) :
0, Oxytocin alone; I, 2×10^{-6} u./ml.;
II, 4×10^{-6} u./ml.; III, 8×10^{-6} u./ml.

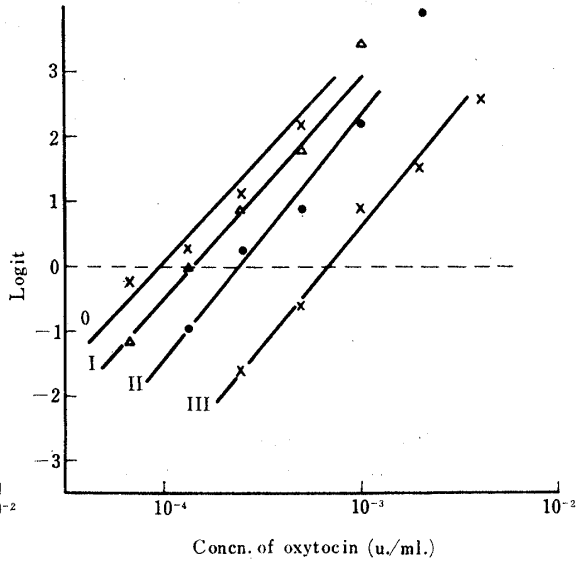

Fig. 3b. Logistic Regression Line of Fig. 3a

4) Antagonism of Peptides to Oxytocin on the Avian Blood Pressure

As ethyl carbobenzoxytyrosyl-tyrosinate inhibited competitively oxytocin on the rat uterus, antagonism on the avian blood pressure might be expected, but it hardly inhibited the depressor effect of oxytocin. Another peptide, di-carbobenzoxycystinyl-di-tyrosine ethyl ester, which was weak inhibitor on the rat uterus, inhibited remarkably the depression of the avian blood pressure by oxytocin. Ten minutes after the injection of di-carbobenzoxycystinyl-di-tyrosine ethyl ester, it inhibited 36 per cent of the response by oxytocin 0.02 u./kg., and after 60 min. it inhibited about 50 per cent of the response by oxytocin 0.02 u./kg. (Fig. 4).

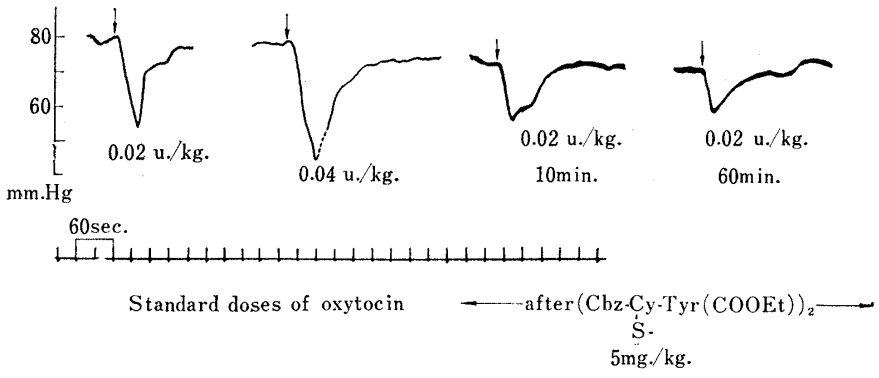

Fig. 4. Antagonistic Action of Di-carbobenzoxycystinyl-di-tyrosine Ethyl Ester to Oxytocin on the Avian Blood Pressure (♂, 2.0 kg.)

5) Inhibitions of the Action of Vasopressin on the Blood Pressure in Dog and Rabbit

Di-carbobenzoxycystinyl-di-tyrosine ethyl ester also inhibited the hypertensive effect of vasopressin in dog and rabbit. This inhibitory action had long duration and after about one hour it inhibited completely the blood pressure elevation (Fig. 5 and Fig. 6).

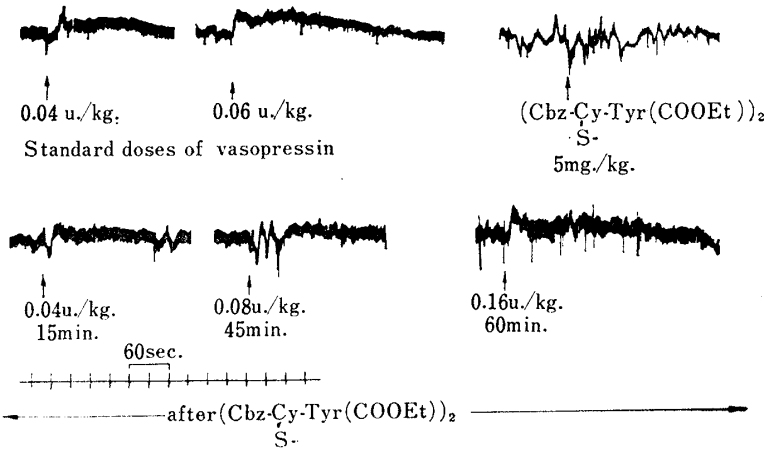

Fig. 5. Antagonistic Action of Di-carbobenzoxycystinyl-di-tyrosine Ethyl Ester to Vasopressin in Dog (♂, 2.0 kg.)

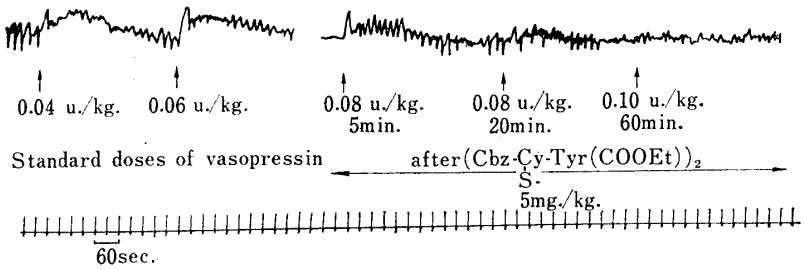

Fig. 6. Antagonistic Action of Di-carbobenzoxycystinyl-di-tyrosine Ethyl Ester to Vasopressin in Rabbit (♂, 1.5 kg.)

Discussion and Conclusions

We had already demonstrated that phenol compounds, such as nitrophenols, estradiol and peptides containing tyrosine inhibited the action of oxytocin on the isolated rat uterus. Ethyl carbobenzoxytyrosyl-tyrosinate inhibited competitively the contraction of oxytocin and was 30.4 times as effective as *p*-nitrophenol on the isolated rat uterus. But it did not inhibit the depressor effects of oxytocin in fowl and the pressor effect of vasopressin in dog and rabbit. On the other hand, di-carbobenzoxycystinyl-di-tyrosine ethyl ester exerted low activity when assayed on the rat uterus, but had some inhibitory activity to oxytocin on the avian blood pressure and to vasopressin on the pressor effects in dog and rabbit. The discrepancy of the effect of the peptides between rat uterus and blood vessel of fowl and of dog or rabbit suggests the difference of active sites of oxytocin or vasopressin receptor between uterus and blood vessel. Recently Cash, *et al.*[6,7] reported that acetylation products of oxytocin and vasopressin inhibit the avian depressor effect of oxytocin and the pressor activity of vasopressin in the rat.

6) R. O. Studer, W. D. Cash : J. Biol. Chem., 238, 657 (1963).
7) W. D. Cash, B. L. Smith : *Ibid.*, 238, 994 (1963).

They said that "recent findings suggest that the receptor sites on blood vessels for the pressor action of the vasopressin are different from the receptors for the vasopressin and oxytocin on uterus and myoepithelial cells." This view agrees with our results.

The authors are indebted to Professor Keijiro Takagi of the University of Tokyo for his advices and encouragement.

Summary

Carbobenzoxylated peptides containing tyrosine inhibited competitively the action of oxytocin on the isolated rat uterus. Among these peptides carbobenzoxy-L-tyrosyl-L-tyrosinate was the most active and 30.4 times as potent as *p*-nitrophenol on the molar basis.

This peptide had non-competitive inhibitions to ACh and barium chloride, but had more active and competitive antagonism to oxytocin when assayed by using their dose-response curves.

On the other hand, it had no inhibition to the avian depressure by oxytocin and to the raising blood pressure by vasopressin.

Di-carbobenzoxy-L-cystinyl-di-L-tyrosine ethyl ester, having a low activity for inhibition of contraction by oxytocin, inhibited about 50 per cent of avian depressure by oxytocin, and almost inhibited the raising blood pressure by vasopressin in dog and rabbit.

(Received April 15, 1964)

[Chem. Pharm. Bull. 12 (8) 877 ~ 888]

UDC 547.574 : 543.253

124. Masaichiro Masui and Hidenobu Ohmori: Studies on Girard Hydrazones. II.*1 Polarographic Reduction Mechanisms of Girard Hydrazones.*2

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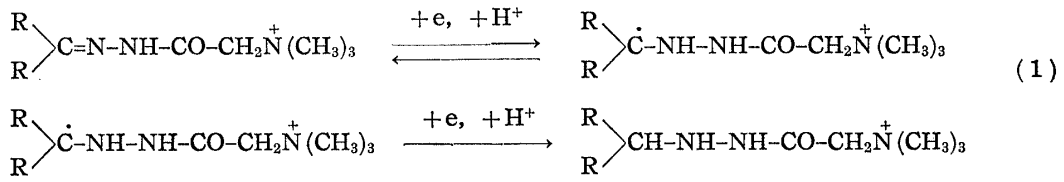
A polarographic reduction process for the Girard-T hydrazones of some aliphatic and alicyclic ketones was proposed by Prelog and Häfliger[1] to be that shown by equation (1), which was supported by Young.[2]



(1)

*1 Part I. J. Chem. Soc. in press.
*2 This paper was read at the 10th Symposium on Polarography in Nagoya, Japan, November 1963.
*3 Hotarugaike, Toyonaka-shi, Osaka-fu (枡井雅一郎, 大森秀信).
1) V. Prelog, O. Häfliger: Helv. Chim. Acta, **32**, 2088 (1949).
2) J.R. Young: J. Chem. Soc., **1955**, 1516.