# Antagonistic Activities of Derivatives of Cystinyl-tyrosyl-tyrosine to Actions of Oxytocin

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**Abstract** □ Peptides with both a tyrosyl-tyrosine and a cystine or *S*-benzylcysteine residue in the molecule (six peptides of cystinyl-tyrosyl-tyrosine derivatives and five peptides of *S*-benzylcysteinyl-tyrosyl-tyrosine derivatives) were synthesized, purified, and evaluated for antagonistic properties of oxytocin. Almost all the peptides exhibited antagonistic activities to the action of oxytocin on isolated rat uterus. The most active peptide was *N*-dicarbobenzoxy-L-cystinyl-di-(L-tyrosyl-L-tyrosine) with $pA_2$ of 6.06. This peptide also showed the inhibition of the responses to oxytocin on rat uterus *in situ* and on avian blood pressure. *N*-Carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine with $pA_2$ of 5.78 did not have the inhibitory actions of oxytocin *in situ*.

**Keyphrases** □ Oxytocin activity antagonists—peptides □ Cystinyl-tyrosyl-tyrosine derivatives—synthesis, oxytocin antagonists □ *S*-Benzyl-cysteinyl-tyrosyl-tyrosine derivatives—synthesis, oxytocin antagonists □ Peptides—as oxytocin antagonists

In a previous paper (1), it was reported that there are two types of oxytocin antagonists. One type consists of phenolic compounds such as peptides containing tyrosine. The other type is L-cystine diethylester, whose action may be related to the disulfide bond of cystine in the oxytocin molecule. Of the antagonists tested by Ishida and Hara (2), *N*-carbobenzoxy-L-tyrosyl-L-tyrosine ethylester (Cbz-Tyr-TyrOEt) was the most potent antagonist of the action of oxytocin on isolated rat uterus; L-cystine diethylester, which has a disulfide bond in the molecule, was a weaker inhibitor. Overweg *et al.* (3) recently reported that *S*-benzyl-tetrapeptide and *S*-benzyl-octapeptide, acyclic oxytocin intermediates, were antagonists of the uterotonic action of the hormone. Both of these peptides have a *S*-benzyl-cysteinyl residue, and the octapeptide also has a tyrosine residue. These results suggest that peptides with both a tyrosyl-tyrosine and a cystine or *S*-benzylcysteine residue in the molecule should be even more potent. The studies reported in this paper were directed to these possibilities.

It was found that *N*-dicarbobenzoxy-L-cystinyl-di-(L-tyrosyl-L-tyrosine) was more antagonistic than Cbz-Tyr-TyrOEt to the action of oxytocin on isolated rat uterus and inhibited the action of oxytocin on rat uterus *in situ* and the avian vasodepressors. *N*-Carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine was almost as antagonistic as Cbz-Tyr-TyrOEt to the action of the hormone on isolated rat uterus.

**Table I**—Physicochemical Properties of Peptides

| Peptide | Melting Point | $[\alpha]_D^t$ (c, Solvent) | Formula | Analysis, % Calcd. | Analysis, % Found | $R_f$(Solvent[a]) |
|---|---|---|---|---|---|---|
| (Cbz-Cys-Tyr-TyrOEt)$_2$ | 173° | −84.0[13] (1, DMF)[b] | $C_{62}H_{68}N_6O_{16}S_2$ | C, 61.17<br>H, 5.63<br>N, 6.90 | C, 61.32<br>H, 6.04<br>N, 6.87 | 0.65(a),0.96(c), 0.98(e) |
| (Cys-Tyr-TyrOEt)$_2$ | 206–207° | −60.8[13] (0.5, DMF) | $C_{46}H_{56}N_6O_{12}S_2$ | C, 58.21<br>H, 5.95<br>N, 8.86 | C, 57.48<br>H, 6.20<br>N, 8.89 | 0.72(c) |
| (Cbz-Cys-Tyr-TyrOH)$_2$ | 183–185° | −41.2[18] (0.5, EtOH) | $C_{58}H_{60}N_6O_{16}S_2$ | C, 59.99<br>H, 5.21<br>N, 7.24 | C, 59.59<br>H, 5.56<br>N, 7.18 | 0.55(e) |
| (Cbz-Cys-Tyr-TyrNH$_2$)$_2$ | 207–210° | −95.6[18] (1, DMF) | $C_{58}H_{62}N_8O_{14}S_2$ | C, 60.09<br>H, 5.39<br>N, 9.67 | C, 59.26<br>H, 5.65<br>N, 9.81 | 0.10(a) |
| Cbz-Cys·Bz-Tyr-TyrOEt | 156.5–157° | −19.7[18] (2, EtOH) | $C_{38}H_{41}N_3O_8S$ | C, 65.22<br>H, 5.91<br>N, 6.01 | C, 65.01<br>H, 6.14<br>N, 5.76 | 0.92(d) |
| Cbz-Cys·Bz-Tyr-TyrOH | 188–189° | −12.0[13] (0.5, EtOH) | $C_{36}H_{37}N_3O_8S$ | C, 64.36<br>H, 5.55<br>N, 6.26 | C, 64.22<br>H, 5.73<br>N, 6.00 | 0.74(d) |
| (Cys-Tyr-TyrOH)$_2$·2H$_2$O | 168° | −53.4[13] (1, DMF) | $C_{42}H_{52}N_6O_{14}S$ | C, 54.30<br>H, 5.65<br>N, 9.05 | C, 54.50<br>H, 5.59<br>N, 9.18 | 0.65(b),0.46(c) |
| Cbz-Cys·Bz-Tyr-TyrNH$_2$ | 204° | −33.8[13] (1, DMF) | $C_{36}H_{38}N_4O_7S$ | C, 64.46<br>H, 5.71<br>N, 8.35 | C, 64.38<br>H, 5.79<br>N, 8.38 | 0.45(a) |
| Cys·Bz-Tyr-TyrOEt·H$_2$O | 157° | −12.0[13] (1, EtOH) | $C_{30}H_{37}N_3O_7S$ | C, 61.73<br>H, 6.39<br>N, 7.20 | C, 61.40<br>H, 6.47<br>N, 7.65 | 0.75(a) |
| Cys·Bz-Tyr-TyrOH·HBr | 148° | +22.6[18] (1, EtOH) | $C_{28}H_{32}BrN_3O_6S$ | C, 54.37<br>H, 5.78<br>N, 6.79 | C, 54.51<br>H, 5.54<br>N, 6.64 | 0.77(b) |
| Cys·Bz-Tyr-TyrNH$_2$ | 148° | +17.4[13] (1, DMF) | $C_{28}H_{32}N_4O_5S$ | C, 62.66<br>H, 6.01<br>N, 10.44 | C, 62.15<br>H, 6.16<br>N, 10.10 | 0.13(a) |

[a] Developing solvents for TLC (v/v): (a) benzene–acetone (1:1); (b) *n*-BuOH–AcOH–H$_2$O (4:1:2); (c) *n*-BuOH–AcOH–H$_2$O (40:5:50); (d) *n*-BuOH–AcOH–pyridine–H$_2$O (4:1:1:2); and (e) *n*-BuOH–pyridine–H$_2$O (40:5:50). [b] DMF = dimethylformamide.

Table II—Comparison of Inhibitory Activities of Peptides to Uterotonic Response of Oxytocin on Isolated Rat Uterus[a]

| Peptide | Concentration, $M$ | $pA_2$ | Average of All Values |
|---|---|---|---|
| (Cbz-Cys-Tyr-TyrOEt)$_2$ | $10^{-5}$ | 5.06 ± 0.13 (6) | 5.08 ± 0.07 (12) |
| | $3 \times 10^{-5}$ | 5.09 ± 0.07 (6) | |
| (Cys-Tyr-TyrOEt)$_2$ | $10^{-5}$ | 5.41 ± 0.10 (8) | 5.54 ± 0.09 (18) |
| | $3 \times 10^{-5}$ | 5.67 ± 0.13 (8) | |
| (Cbz-Cys-Tyr-TyrOH)$_2$ | $3 \times 10^{-6}$ | 6.09 ± 0.17 (6) | 6.06 ± 0.13 (22) |
| | $10^{-5}$ | 5.95 ± 0.16 (8) | |
| | $3 \times 10^{-5}$ | 6.15 ± 0.17 (8) | |
| (Cys-Tyr-TyrOH)$_2$ | $10^{-4}$ | No inhibition | |
| (Cbz-Cys-Tyr-TyrNH$_2$)$_2$ | $10^{-5}$ | 4.80 ± 0.11 (4) | 4.83 ± 0.07 (8) |
| | $3 \times 10^{-5}$ | 4.86 ± 0.10 (4) | |
| Cbz-Cys·Bz-Tyr-TyrOEt | $10^{-4}$ | No inhibition | |
| Cys·Bz-Tyr-TyrOEt | $10^{-5}$ | 5.20 ± 0.13 (4) | 5.12 ± 0.11 (8) |
| | $3 \times 10^{-5}$ | 5.04 ± 0.18 (4) | |
| Cbz-Cys·Bz-Tyr-TyrOH | $10^{-5}$ | 5.70 ± 0.10 (4) | 5.78 ± 0.06 (8) |
| | $3 \times 10^{-5}$ | 5.85 ± 0.07 (4) | |
| Cys·Bz-Tyr-TyrOH | $10^{-5}$ | 4.97 ± 0.10 (4) | 5.01 ± 0.07 (8) |
| | $3 \times 10^{-5}$ | 5.05 ± 0.10 (4) | |
| Cbz-Cys·Bz-Tyr-TyrNH$_2$ | $3 \times 10^{-5}$ | 4.76 ± 0.11 (4) | 4.79 ± 0.07 (8) |
| | $10^{-4}$ | 4.82 ± 0.11 (4) | |
| Cys·Bz-Tyr-TyrNH$_2$ | $3 \times 10^{-5}$ | 4.62 ± 0.17 (4) | 4.68 ± 0.10 (8) |
| | $10^{-4}$ | 4.74 ± 0.12 (4) | |
| Cbz-Tyr-TyrOEt | $10^{-5}$ | 5.70 ± 0.14 (8) | 5.74 ± 0.09 (16) |
| | $3 \times 10^{-5}$ | 5.79 ± 0.11 (8) | |

[a] Mean ± $SE$, number of experiments in parentheses.

## EXPERIMENTAL

**Synthetic Procedure**—The antagonists used were synthesized in this laboratory. *N*-Dicarbobenzoxy-L-cystinyl-di-(L-tyrosyl-L-tyrosine ethylester), (Cbz-Cys-Tyr-TyrOEt)$_2$, was synthesized by coupling 1 mole of *N*-dicarbobenzoxy-L-cystine (5.08 g.) and 2 moles of L-tyrosyl-L-tyrosine ethylester hydrobromide (4) (9.06 g.) by *N*,*N*-dicyclohexylcarbodiimide (yield 9.8 g.). From this peptide ester protected by carbobenzoxy radicals, the next three peptides were obtained as follows: L-cystinyl-di-(L-tyrosyl-L-tyrosine ethylester), (Cys-Tyr-TyrOEt)$_2$, was obtained by decarbobenzoxylation with 25% HBr in glacial acetic acid; *N*-dicarbobenzoxy-L-cystinyl-di-(L-tyrosyl-L-tyrosine), (Cbz-Cys-Tyr-TyrOH)$_2$, was obtained by hydrolysis with 2 *N* NaOH in methanol; and *N*-dicarbobenzoxy-L-cystinyl-di-(L-tyrosyl-L-tyrosine amide), (Cbz-Cys-Tyr-TyrNH$_2$)$_2$, was obtained by ammonolysis in NH$_3$-saturated methanol.

*N*-Carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine ethylester, Cbz-Cys·Bz-Tyr-TyrOEt, was prepared from *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosine hydrazide (6.28 g.) and L-tyrosine ethylester hydrochloride (3.04 g.) by the azide method (yield 4.2 g.). Alkaline hydrolysis gave *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine, Cbz-Cys·Bz-Tyr-TyrOH, from which L-cystinyl-di-(L-tyrosyl-L-tyrosine), (Cys-Tyr-TyrOH)$_2$, was obtained by cleavage with sodium in liquid ammonia and then by oxidation. *N*-Carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine amide, Cbz-Cys·Bz-Tyr-TyrNH$_2$, was prepared by ammonolysis from Cbz-Cys·Bz-Tyr-TyrOEt. *S*-Benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine ethylester (Cys·Bz-Tyr-TyrOEt), *S*-benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine (Cys·Bz-Tyr-TyrOH), and *S*-benzyl-L-cysteinyl-L-tyrosyl-L-tyrosine amide (Cys·Bz-Tyr-TyrNH$_2$) were obtained by treatment of the corresponding *N*-carbobenzoxypeptides with 25% HBr in glacial acetic acid.

The hydrobromide salts of (Cys-Tyr-TyrOEt)$_2$, Cys·Bz-Tyr-TyrOEt, and Cys·Bz-Tyr-TyrNH$_2$ were neutralized with sodium bicarbonate solution and free bases obtained were recrystallized from alcohol.

The structures of the peptides were confirmed by elemental analysis and TLC. Physicochemical properties of these peptides are given in Table I.

## PHARMACOLOGICAL PROCEDURES

**Isolated Rat Uterus**—Experiments were made on strips of smooth muscle suspended in a bath containing 10 ml. of aerated physiological saline solution at 28°. Isotonic recordings were made with a light lever on a smoked drum. The uterine horns were taken from virgin rats of about 200-g. weight, preferably in the stage of natural dioestrus or dioestrus after ovariectomy. These were suspended in the physiological saline solution of Kumagai *et al.* (5) which had the following composition (in grams per liter): NaCl, 8.8; KCl, 0.4; $CaCl_2$, 0.04; $MgCl_2$, 0.018; $NaHCO_3$, 0.4; $KH_2PO_4$, 0.08; and glucose, 0.5. It contains a lower concentration of calcium chloride than does de Jalon's but does contain magnesium chloride. Since antagonists were insoluble in water, they were used as emulsions with gum arabic. Some acidic peptides were neutralized with sodium bicarbonate, since acidic conditions greatly inhibit the uterotonic action of oxytocin (6). Synthetic oxytocin[1] was used for the assay.

The cumulative dose procedure was used as described by van Rossum (7). The presence of a competitive antagonist results in a parallel shift of the log dose–response curve for oxytocin to higher concentrations. The $pA_2$ value for competitive inhibition was calculated from the following equation, according to the method of Schild (8, 9) and van Rossum (7), from the shift of the curve:

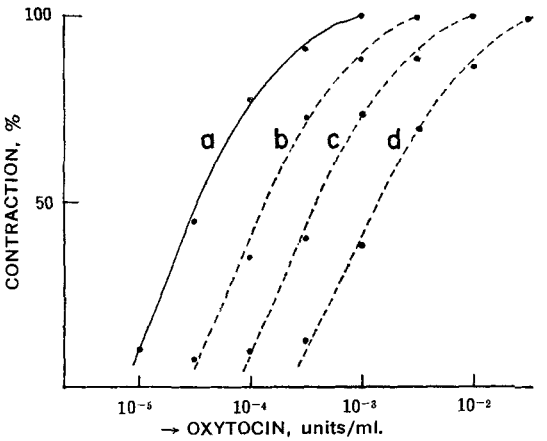


**Figure 1**—*Typical example of cumulative log dose–response curves of oxytocin in the presence of (Cbz-Cys-Tyr-TyrOH)$_2$ on isolated rat uterus. Key: Curve a, oxytocin alone; and Curves b, c, and d, in the presence of 3 × $10^{-6}$, $10^{-5}$, and 3 × $10^{-5}$ M of inhibitor, respectively.*

[1] Syntocinon, Sandoz AG.

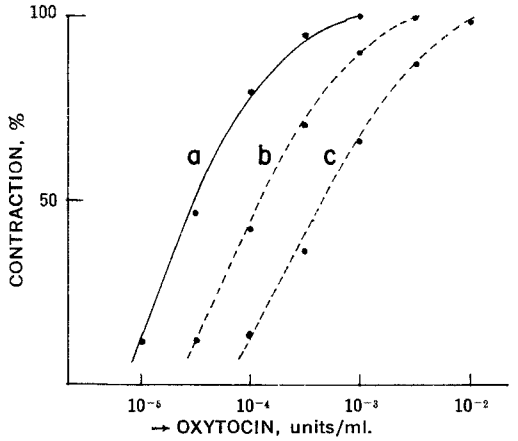


**Figure 2**—*Typical example of cumulative log dose–response curves of oxytocin in the presence of Cbz-Cys·Bz-Tyr-TyrOH on isolated rat uterus. Key: Curve a, oxytocin alone; and Curves b and c, in the presence of $10^{-5}$ and $3 \times 10^{-5}$ M of inhibitor, respectively.*

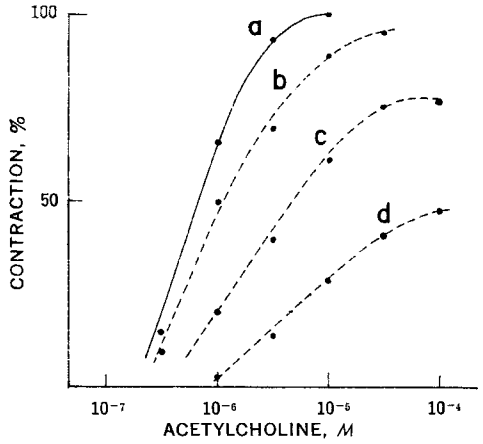


**Figure 3**—*Typical example of cumulative log dose–response curves of acetylcholine in the presence of Cbz-Cys·Bz-Tyr-TyrOH on isolated rat uterus. Key: Curve a, acetylcholine alone; and Curves b, c, and d, in the presence of $3 \times 10^{-5}$, $10^{-4}$, and $3 \times 10^{-4}$ M of inhibitor, respectively. Note the decrease of maximum height of the contractions induced by acetylcholine. This indicates noncompetitive antagonism between acetylcholine and the inhibitor.*

$pA_2 = pA_x + \log(x - 1)$, where $pA_x$ is the negative logarithm of the concentration of the antagonist causing the shift of $x$. The $pD_2'$ value for noncompetitive antogonism is the negative logarithm of the molar concentration of antagonist that may cause a 50% depression of the maximum concentration of antagonist (7). All concentrations, unless otherwise stated, are expressed in moles in a liter ($M$) for antagonists.

**Rat Uterus *In Situ***—Rats of about 200 g., 1–2 weeks after ovariectomy, received 5 mcg./100 g. of estradiol benzoate each day for 2 days before the experiments. The animals were anesthetized with urethan given in two dosages (first, 100 mg./100 g. i.p., and second, 50 mg./100 g. s.c.). Atropine, 0.1 mg./100 g. s.c., was also administered. Polyethylene tubing filled with normal saline was inserted through a cut in the wall into the lumen of the horn near the cervix, and pressures were recorded from it with a Nihon Kohden Mp-4 pressure transducer and a polygraph, since uterine contractions result in changes in the intrauterine pressure.

**Avian Blood Pressure**—Leghorn cocks of about 2 kg. were anesthetized with sodium phenobarbital, 200 mg./kg. s.c. The arterial pressure was recorded on a smoked drum with a mercury manometer. Oxytocin and antagonists were injected into the femoral vein.

## RESULTS

**Isolated Rat Uterus**—Almost all the peptides with one or two tyrosyltyrosine residues exhibited antagonistic effects on the uterotonic action of oxytocin as shown in Table II. However, two peptides, (Cys-Tyr-TyrOH)$_2$ and Cbz-Cys·Bz-Tyr-TyrOEt, did not show any antagonistic action to oxytocin even at high concentrations of $10^{-4}$ $M$. The $pA_2$ values were calculated from the parallel shifts of the log dose–response curve of oxytocin. The most active antagonist among these peptides was (Cbz-Cys-Tyr-TyrOH)$_2$, which was more potent than Cbz-Tyr-TyrOEt (2). The log dose–response curves of the rat uterus to oxytocin in the presence of the peptide at concentrations of $3 \times 10^{-6}$, $10^{-5}$, and $3 \times 10^{-5}$ $M$ (Fig. 1, Curves b, c, and d) were parallel to that of oxytocin alone (Curve a); and at these concentrations there was no decrease in the maximal response. The $pA_2$ value of (Cbz-Cys-Tyr-TyrOH)$_2$ was calculated as 6.02. The second most active one was Cbz-Cys·Bz-Tyr-TyrOH with a $pA_2$ value of 5.78 (Fig. 2).

It was concluded that (Cbz-Cys-Tyr-TyrOH)$_2$ was the most specific antagonist of oxytocin; at higher concentrations of $3 \times 10^{-5}$, $10^{-4}$, and $3 \times 10^{-4}$ $M$, it did not inhibit uterine contractions induced by acetylcholine or bradykinin. As shown in Table III, Cbz-Tyr-TyrOEt inhibited both acetylcholine and bradykinin noncompetitively at over $3 \times 10^{-5}$ $M$, and Cbz-Cys·Bz-Tyr-TyrOH only inhibited acetylcholine noncompetitively at over $10^{-4}$ $M$ (Fig. 3) but had no effect on bradykinin.

**On Rat Uterus *In Situ***—Most of the rat preparations described under experimental methods were suitable for assay of antagonism to oxytocin because they did not contract spontaneously during repeated oxytocin infusion. On these preparations, high dosages of oxytocin, 100 and 200 milliunits/kg., had to be used for inducing the uterotonic contractions. Figure 4 shows recordings in a typical experiment. The rat was given two control dosages of oxytocin, 100 and 200 milliunits/kg. When 2 mg./kg. of (Cbz-Cys-Tyr-TyrOH)$_2$ was infused, the contraction resulting from injection of 100 milliunits/kg. of oxytocin was nearly completely suppressed. Recovery was observed in less than 15 min. after cessation of infusion of the inhibitor. A dosage of 4 mg./kg. of the inhibitor also suppressed the contractions due to 200 milliunits/kg. of oxytocin. Some uteri of normal rats, without ovariectomy, in the stage of oestrus or dioestrus, did not show spontaneous contractions at the beginning of the experiments, but antagonism of the peptide to the action of oxytocin was also observed in these animals. After repeated infusions of oxytocin the uteri gradually developed spontaneous contractions during which the inhibitory activity of the peptide could not be estimated. An example of the latter part of an experiment is shown on Fig. 5. Dosages of 5–20 milliunits/kg. of oxytocin were used for the assay. Among the antagonistic peptides tested, (Cbz-Cys-Tyr-TyrOH)$_2$ was the most active and (Cys-Tyr-TyrOEt)$_2$·2HBr, another of the cystinyl derivatives, showed weak inhibition. Other peptides tested, such as Cbz-Cys·Bz-Tyr-TyrOH and Cbz-Tyr-TyrOEt did not inhibit the uterotonic action of oxytocin *in situ*.

**On Avian Blood Pressure**—(Cbz-Cys-Tyr-TyrOH)$_2$ also inhibited the effect of oxytocin in reducing the blood pressure of Leghorn cocks as shown in Fig. 6. (Cys-Tyr-TyrOEt)$_2$·2HBr also caused weak

**Table III**—Inhibitory Activities of Some Active Peptides to Oxytocin, Acetylcholine, and Bradykinin on Isolated Rat Uterus[a]

| Antagonist and Concentration ($M$) | To Oxytocin $pA_2$ | To Oxytocin $pD_2'$ | To Acetylcholine $pA_2$ | To Acetylcholine $pD_2'$ | To Bradykinin $pA_2$ | To Bradykinin $pD_2'$ |
|---|---|---|---|---|---|---|
| (Cbz-Cys-Tyr-TyrOH)$_2$ | | | | | | |
| $3 \times 10^{-6}$ | 6.09 | (−) | | | | |
| $10^{-5}$ | 5.95 | (−) | | | | |
| $3 \times 10^{-5}$ | 6.16 | (−) | | | | |
| $10^{-4}$ | | | (−) | (−) | (−) | (−) |
| Cbz-Cys·Bz-Tyr-TyrOH | | | | | | |
| $10^{-5}$ | 5.70 | (−) | | | | |
| $3 \times 10^{-5}$ | 5.85 | (−) | | | | |
| $10^{-4}$ | | | | 3.40 | (−) | (−) |
| $3 \times 10^{-4}$ | | | | 3.50 | (−) | (−) |
| Cbz-Tyr-TyrOEt | | | | | | |
| $10^{-5}$ | 5.70 | (−) | | (−) | | (−) |
| $3 \times 10^{-5}$ | 5.79 | (—) | | 3.68 | | 3.73 |
| $10^{-4}$ | | | | 3.88 | | 3.85 |

[a] $pA_2$ for competitive inhibition and $pD_2'$ for noncompetitive inhibition.

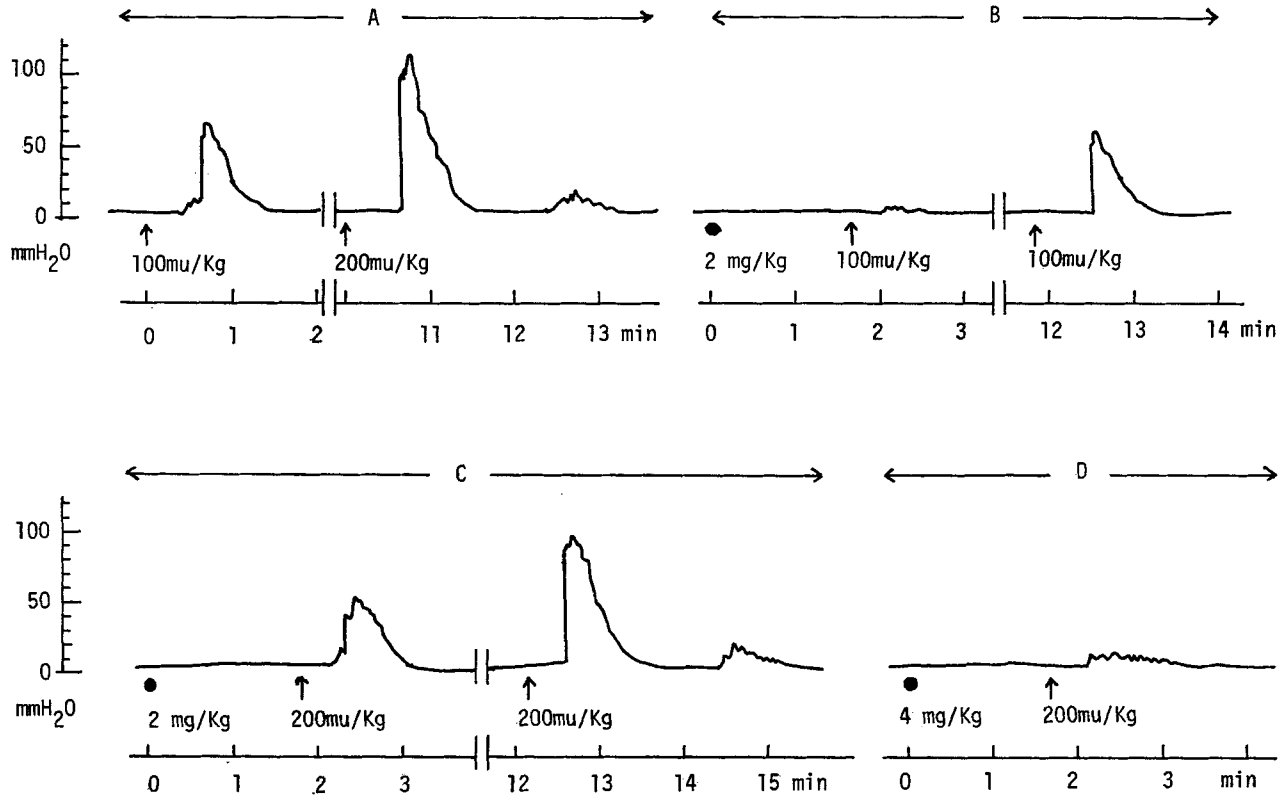


**Figure 4**—*Inhibition by (Cbz-Cys-Tyr-TyrOH)₂ to oxytocin on rat uterus* in situ. *The rat was in the stage of oestrus induced by injection of estradiol benzoate after ovariectomy. Column A = control contractions of two dosages of oxytocin. Columns B and C = inhibition by 2 mg./kg. of the peptide to 100 and 200 milliunits/kg. of oxytocin. Column D = 4 mg./kg. of the peptide to 200 milliunits/kg. of oxytocin. Note that inhibitions by peptide recovered in less than 15 min.*

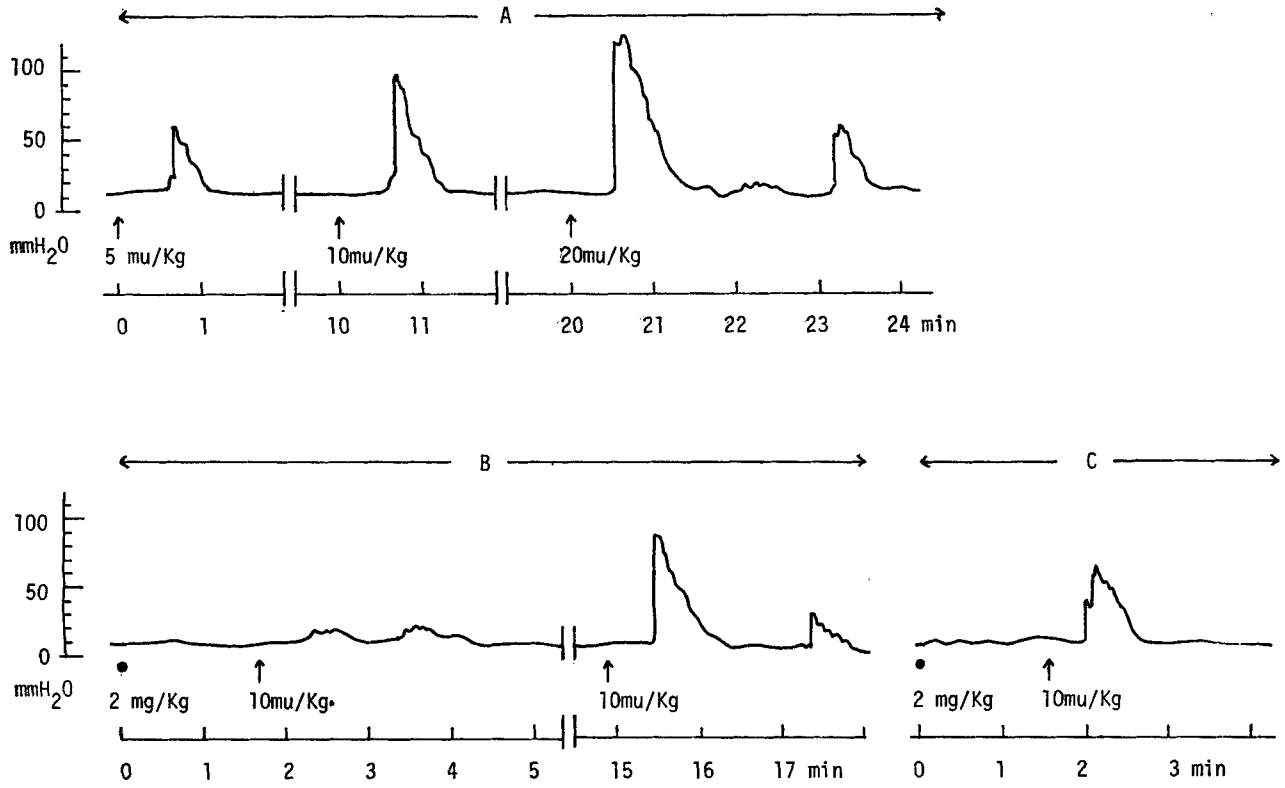


**Figure 5**—*Inhibition by (Cbz-Cys-Tyr-TyrOH)₂ to oxytocin on rat uterus* in situ. *The rat was in the stage of natural oestrus. Column A = control contractions of three dosages of oxytocin. Columns B and C = inhibition by 2 mg./kg. of the peptide to 10 and 20 milliunits/kg. of oxytocin. Note that dosages of oxytocin in natural oestrus for inducing contractions were smaller than those in the rat in Fig. 4.*

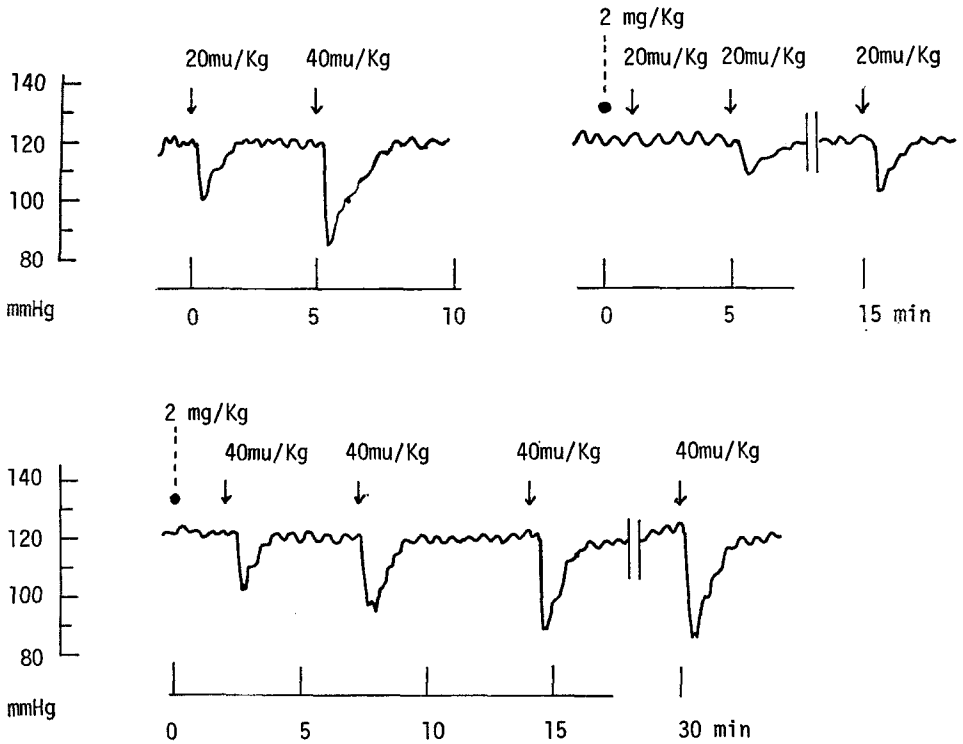


**Figure 6**—*Inhibition by (Cbz-Cys-Tyr-TyrOH)₂ to oxytocin on avian blood pressure. Column A = control blood depressure of two dosages of oxytocin. Columns B and C = inhibition by 2 mg./kg. of the peptide to 20 and 40 milliunits/kg. of oxytocin.*

inhibition, while Cbz-Cys·Bz-Tyr-TyrOH and Cbz-Tyr-TyrOEt had no inhibitory activity on it.

## DISCUSSION

The experiments show that (Cbz-Cys-Tyr-TyrOH)$_2$ is a strong inhibitor of the uterotonic response to oxytocin of rat uterus both *in vitro* and *in situ*, and of the effect of oxytocin on avian blood pressure. The log dose–response analysis, as shown in Fig. 1, indicates that the inhibition of the response to oxytocin in the presence of the antagonist is competitive and reversible. It did not inhibit the response to acetylcholine or bradykinin, or spontaneous uterine contractions either *in vitro* or *in situ*. Chan *et al.* (10) reported that 1-L-penicillamine oxytocin and 1-deaminopenicillamine oxytocin were strong inhibitors of the actions of oxytocin both *in vitro* and *in vivo*. The inhibitory actions of (Cbz-Cys-Tyr-TyrOH)$_2$ to oxytocin might be quite similar qualitatively to those of the penicillamine analogs. But its effect is much weaker because it has been reported that the dose-response curve of oxytocin can be inhibited by 1-L-penicillamine oxytocin at $25 \times 10^{-11}$ and $50 \times 10^{-11}$ *M*.

Overweg *et al.* (3) reported interesting data on the inhibition of the action of oxytocin on isolated rat uterus by acyclic intermediates. Among the peptides they tested, the octapeptides, L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, which contains a tyrosine residue, was found to be a competitive inhibitor of the uterotonic response to oxytocin ($pA_2$ of 5.70). The tetrapeptide with a *S*-benzyl-cysteinyl residue, *S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, is only a competitive inhibitor at low concentrations ($pA_2$ of 4.20). At higher doses of tetrapeptide ($8–10 \times 10^{-4}$ *M*), a factor other than pure competitive inhibition became evident, as indicated by the decrease in the maximal response. The *S*-benzylcysteinyl peptide, Cbz-Cys·Bz-Tyr-TyrOH ($pA_2$ of 5.70), considering the $pA_2$ value, is a more potent inhibitor than the above tetrapeptide, but at higher doses ($10^{-4}$ and $3 \times 10^{-4}$ *M*) it showed an inhibitory response to acetylcholine and not to bradykinin. Furthermore, it does not inhibit there sponse to oxytocin *in situ*. Cbz-Tyr-TyrOEt inhibits the responses to acetylcholine and bradykinin noncompetitively, as shown in Table III. When tested on isolated guinea pig ileum, none of the peptides tested showed the inhibitory responses to acetylcholine, barium chloride, and histamine.

Thus, this study shows that a peptide containing both cystine and tyrosyl-tyrosine residues, (Cbz-Cys-Tyr-TyrOH)$_2$, is a more specific and stronger antagonist of responses to oxytocin than Cbz-Tyr-Tyr-OEt or cystine diethylester.

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