PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **185**, 187–192 (1987)

Long-Acting Oxytocin Antagonists: Effects of 2-D-Stereoisomer Substitution on Antagonistic Potency and Duration of Action[1] (42533)

W. Y. CHAN,[2] TODD W. ROCKWAY,* AND VICTOR J. HRUBY*

*Department of Pharmacology, Cornell University Medical College, New York, New York 10021; and *Department of Chemistry, University of Arizona, Tucson, Arizona 85711*

Abstract. Recently we reported the discovery of a series of 2-*O*-alkyltyrosine- (or 2-*p*-alkylphenylalanine), 4-threonine-, and 8-ornithine-substituted analogs of [1-penicillamine]oxytocin ([Pen^1]OT) which possess prolonged anti-OT activity. In this study, we attempt to improve the potency and the duration of action of this series of OT antagonists by exploring the effects of D-stereoisomer substitution in the 2 position. We compare the *in vitro* anti-OT potency, expressed in pA_2 values, and the duration of *in vivo* inhibitory action, expressed in recovery $t_{1/2}$, of [Pen^1]OT, [Pen^1,Orn^8]OT, [Pen^1,Thr^4]OT, [Pen^1,Tyr(OMe)^2,Thr^4,Orn^8]OT, [Pen^1,Tyr(OEt)^2,Thr^4,Orn^8]OT, [Pen^1,D-Tyr(OEt)^2,Thr^4,Orn^8]OT, [Pen^1,Phe^2,Thr^4]OT, [Pen^1,Phe(Me)^2,Thr^4,Orn^8]OT, [Pen^1,D-Phe(Me)^2,Thr^4,Orn^8]OT, [Pen^1,Phe(Et)^2,Thr^4,Orn^8]OT, and [Pen^1,D-Phe(Et)^2,Thr^4,Orn^8]OT. The results show that modifications of the amino acid in position 2 by alkylation of the aromatic ring and use of D-stereoisomerism produce nonparallel effects on the *in vitro* potency and duration of action of OT antagonists. Time–action curve determinations show that long-acting OT antagonists exhibit delayed peak inhibitory action. Long action is not coupled with high potency in all cases. This dissociation between potency and duration of action gives support to our hypothesis that the potency and duration of action of these peptides may each have different conformational structure requirements.

Recently, we synthesized a series of oxytocin (OT)[3] antagonists which possess prolonged action (1, 2). They were 2-*O*-alkyltyrosine- (or 2-*p*-alkylphenylalanine), 4-threonine-, and 8-ornithine-substituted analogs of [1-penicillamine]oxytocin ([Pen^1]OT). Pharmacological studies on these long-acting OT antagonists showed a dissociation between antagonistic potency and duration of action (2). This finding supports our hypothesis that potency and duration of action may each have different molecular requirements (3–8) and suggests that OT analogs might be designed rationally for potency, specificity, and long action based on conformational considerations. As a continuing effort to elucidate the optimal structure for potent and long-acting OT antagonists, we have explored the effects of D-stereoisomeric substitution in the 2 position. [Pen^1,D-Phe(Me)^2,Thr^4,Orn^8,]OT, [Pen^1,D-Phe(Et)^2, Thr^4,Orn^8]OT, and [Pen^1,D-Tyr(OEt)^2, Thr^4,Orn^8]OT were synthesized. In this paper, we compare the anti-OT potency and the duration of action of the 2-D-isomer-containing analogs with their respective 2-L-isomer-containing compounds.

Materials and Methods. *In vitro oxytocic assays. In vitro* oxytocic assays were performed on isolated uteri from rats (Wistar) in natural estrus, as described in one of our previous papers (6), with the use of a Mg^{2+}-free van Dyke–Hasting solution. Antagonistic potencies were measured in this *in vitro* system and were expressed in pA_2 values by the method of Schild (9). Synthetic OT was used as the agonist.

In vivo oxytocic assays. In vivo oxytocic or anti-OT activities were determined in urethane-anesthetized 21- or 22-day pregnant rats according to the method introduced in our laboratory (10). In brief, the technique was an isometric contraction recording of a segment of the uterus *in situ*. Uterine contractions were induced by iv injections of OT and recorded on a polygraph via a force-displacement transducer. The standard OT doses used in

[1] Supported by NIH Grants HD-20839 and AM-17420 and a NSF grant.

[2] To whom reprint requests and correspondence should be addressed: Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

[3] Abbreviations used: OT, oxytocin; Pen, penicillamine; Tyr(OMe), *O*-methyl-Tyr; Tyr(OEt), *O*-ethyl-Tyr; Phe(Me), *p*-methyl-Phe; Phe(Et), *p*-ethyl-Phe.

0037-9727/87 $1.50

the experiment were between 50 and 80 mU/rat. The contractile response to OT injection was expressed by the area under the contractile curve for the 10-min interval immediately following drug administration. After the response to the OT dose was established, an appropriate dose (μg) of the OT antagonist was selected and injected 20–30 sec preceding the standard OT dose so that the OT response was reduced by 50–80%. Time–action of the inhibitory response was measured by monitoring the recovery of OT response. OT injections were repeated at 30-min intervals. The time–action curves were plotted and the maximal inhibition of OT response was determined. The time for 50% recovery from the maximal inhibition was determined by interpolation from the time–action curves. This time is designated as the recovery $t_{1/2}$. In control experiments, the contractile responses to the standard OT injections at 30-min intervals were relatively reproducible over a 2-hr period. There was no evidence of desensitization to OT over this period. On the contrary, in general the contractile response to OT gradually increased following repeated injections. This increased OT sensitivity might have underestimated the recovery $t_{1/2}$ of some OT antagonists with $t_{1/2}$ longer than 90 min.

Statistical analysis of data. Bioassay values were presented as means ± SEM and analyzed by analysis of variance. Significant differences between sample means of the L- and D-isomers were analyzed by Student's *t* test, both tails at 0.05 *P* level.

Materials. The OT antagonists used in this study were synthesized in our laboratory. They were [Pen^1]OT, [Pen^1,Orn^8]OT, [Pen^1,Thr^4]OT, [Pen^1,Tyr(OMe)^2,Thr^4,Orn^8]OT, [Pen^1,Tyr(OEt)^2,Thr^4,Orn^8]OT, [Pen^1,DTyr(OEt)^2,Thr^4,Orn^8]OT,[Pen^1,Phe^2,Thr^4]OT, [Pen^1,Phe(Me)^2,Thr^4,Orn^8]OT, [Pen^1,D-Phe(Me)^2,Thr^4,Orn^8]OT,[Pen^1,Phe(Et)^2,Thr^4,Orn^8]OT, and [Pen^1,D-Phe(Et)^2,Thr^4,Orn^8]OT. Details of the synthesis of the peptides and their physicochemical properties will be reported elsewhere (in preparation). The OT used was Pitocin (Parke–Davis, Morris Plains, NJ).

Results. *Time–action of OT antagonists.* The 2-alkylated and 8-ornithine-substituted OT analogs exibited prolonged anti-OT action in both *in vitro* assays on the isolated nonpregnant rat uterus and *in vivo* assays in the pregnant rat.

In the *in vitro* assays, these alkylated long-acting OT antagonists, unlike other short-acting OT antagonists which can be washed out readily from the tissue bath, required repeated washouts over 15 to 30 min to regain the control OT response.

Time–action studies in the pregnant rat showed that 8-ornithine substitution alone produced an analog with prolonged action. Combination of 2-*O*-alkyltyrosine (or 2-*p*-alkylphenylalanine) and 8-ornithine substitution produced analogs with even more prolonged action as was reported in our prior paper (2). OT antagonists with a response recovery $t_{1/2}$ longer than 100 min developed their peak inhibition over a period of 30 min following their iv injections. Figure 1 illustrated the three patterns of anti-OT time–action: (i) immediate and short acting, as represented by [Pen^1]OT and [Pen^1,Phe^2,Thr^4]OT; (ii) immediate and moderately long acting, as represented by [Pen^1,Orn^8]OT and [Pen^1,Tyr(OEt)^2,Thr^4,Orn^8]OT; and (iii) very long acting with gradual peak action, as represented by [Pen^1,Tyr(OMe)^2,Thr^4,Orn^8]OT,[Pen^1,Phe(Me)^2,Thr^4,Orn^8]OT, [Pen^1,D-Phe(Me)^2,Thr^4,Orn^8]OT, and [Pen^1,D-Phe(Et)^2,Thr^4,Orn^8]OT. In this experimental protocol, the peak anti-OT response produced by the OT antagonist dose administered was taken as the 0% recovery of inhibitory response. The dose of OT antagonist injected was that required to produce a 50–80% inhibition of the response to the standard OT dose.

Anti-OT potency and duration of antagonistic action. Anti-OT potency was determined on isolated nonpregnant rat uteri and expressed in pA_2 values. Duration of antagonistic action was determined in 21- or 22-day pregnant rats. The recovery $t_{1/2}$ (the time for 50% recovery from the peak inhibition of the standard OT response) was determined by interpolation from the time–action curve as shown in Fig. 1. The *in vitro* pA_2 and the *in vivo* recovery $t_{1/2}$ values of the OT antagonists are shown in Table I. Values for the L-isomers have been reported previously (2).

The *in vitro* pA_2 values for a number of very long-acting OT antagonists could not be determined precisely due to the difficulty of washing out the inhibitory effect and shifting

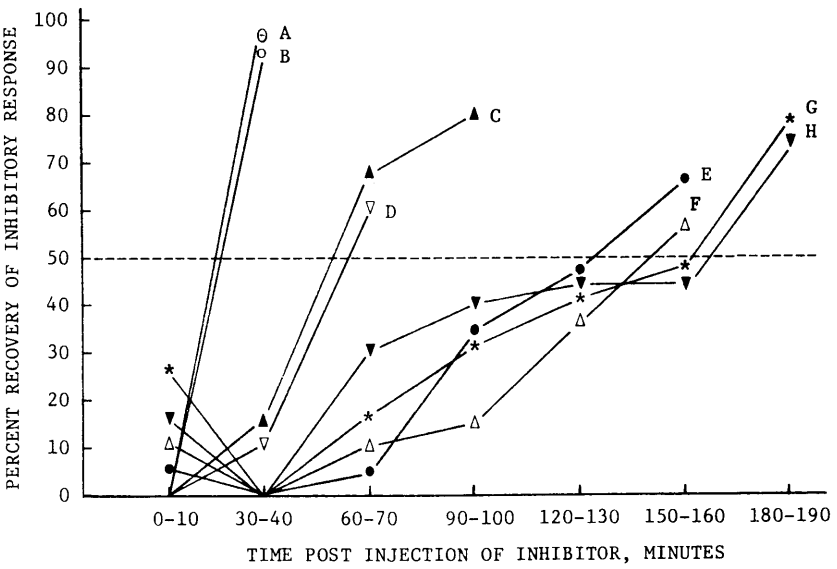

FIG. 1. Time-action of long-acting OT antagonists. Recovery of contractile responses to OT in pregnant rats following injection of an OT antagonist. The standard OT doses were 50–80 mU/rat injected at 30-min intervals. Contractile responses were expressed by the area under the contractile curve for the 10-min interval immediately following OT injection. The OT antagonist was injected once at minus 20–30 sec at a dose level that produced 50–80% inhibition of the standard OT response. Each curve represents the average values from at least four 21- or 22-day pregnant rats. The OT response immediately preceding the OT antagonist injection was taken as 100%. 0% recovery of inhibitory response represented the maximal inhibitory response. A = [Pen^1]OT. B = [Pen^1,Phe^2,Thr^4]OT. C = [Pen^1,Orn^8]OT. D = [Pen^1,Tyr(OEt)^2,Thr^4,Orn^8]OT. E = [Pen^1,Tyr(OMe)^2,Thr^4,Orn^8]OT. F = [Pen^1,D-Phe(Me)^2,Thr^4,Orn^8]OT. G = [Pen^1,D-Phe(Et)^2,Thr^4,Orn^8]OT. H = [Pen^1,Phe(Me)^2,Thr^4,Orn^8]OT.

the control OT response because of the long time lapse (>30 min). For these OT antagonists, the pA_2 values given are regarded as best estimates.

In vivo recovery $t_{1/2}$ values were compared based on approximately equieffective doses of the OT antagonists which produced a 50–80% inhibition of the contractile response to the standard dose of OT in the pregnant rat.

It is apparent from the data presented in Table I that relative to [Pen^1]OT and [Pen^1,Phe^2,Thr^4]OT, the prototypic OT antagonists, substitution of ornithine in the 8 position and/or in combination with 2-alkylation increased duration of action but not potency while substitution of a D-amino acid residue in the 2 position enhanced potency but not duration of action of the OT antagonists.

Discussion. OT is the most potent oxytocic agent known to clinical medicine. Recent laboratory and clinical observations (11–13) suggest that OT may play a pivotal role in the initiation and maintenance of labor. Specific OT antagonists, thus, may be effective as tocolytic agents for the prevention of preterm labor.

A number of *in vivo* active OT antagonists have been synthesized over the years in our laboratory and others (3, 4, 10, 14, 15). OT peptides in general have a very short duration of action because of their rapid clearance rate. To be useful as tocolytic agents for preterm labor, more specific, potent, and, in particular, longer-acting antagonists will be required. As a result of our continuing effort to develop more potent and long-acting OT antagonists, we have recently discovered a new series of 2-*O*-alkyltyrosine- (or 2-*p*-alkylphenylalanine), 4-threonine-, 8-ornithine-substituted analogs of [Pen^1]OT which possess prolonged anti-OT activity (1, 2). In this study, we attempted to improve the potency and duration of action of this series of OT antagonists by exploring

TABLE I. ANTI-OT POTENCY AND DURATION OF ACTION OF OT ANTAGONISTS

OT antagonists	*In vitro* pA_2	*In vivo* relative potency and recovery $t_{1/2}$	
		Dose range for 50–80% inhibition of OT response (μg)	Recovery $t_{1/2}$ (min)
[Pen^1]OT	6.86 ± 0.03	10	~20
[Pen^1,Orn^8]OT	6.75 ± 0.07	10–20	50 ± 4
[Pen^1,Thr^4]OT ([Pen^1,Tyr^2,Thr^4]OT)	7.55 ± 0.04	5.0	~20
[Pen^1,Tyr(OMe)^2,Thr^4,Orn^8]OT	~7.3[a]	3.5–5.0	130 ± 16
[Pen^1,Tyr(OEt)^2,Thr^4,Orn^8]OT	5.32 ± 0.02	6.0–8.0	55 ± 12[b]
[Pen^1,D-Tyr(OEt)^2,Thr^4,Orn^8]OT	7.36 ± 0.07	4.0	45 ± 2[b]
[Pen^1,Phe^2,Thr^4]OT	7.67 ± 0.02	5.0	~20
[Pen^1,Phe(Me)^2,Thr^4,Orn^8]OT	~7.3[a]	3.0–4.0	160 ± 14[b]
[Pen^1,D-Phe(Me)^2,Thr^4,Orn^8]OT	~7.5[a]	2.5	148 ± 11[b]
[Pen^1,Phe(Et)^2,Thr^4,Orn^8]OT	5.80 ± 0.03	40–50	110 ± 9[b]
[Pen^1,D-Phe(Et)^2,Thr^4,Orn^8]OT	~7.5[a]	3.5–5.0	155 ± 22[b]

Note. Values shown are means ± SEM. Values for the L-isomers have been reported previously (2). For pA_2, n = at least 6. For recovery $t_{1/2}$, n = at least 4.

[a] Estimated values only. See text for explanation.

[b] Not significantly different from its stereoisomer value.

the effects of substitution of D-amino acid stereoisomers in the 2 position. D-Tyr substitution at position 2 of vasopressin antagonists has been shown by Manning *et al.* (16) to increase antagonistic potency. As shown in Table I, D-Tyr(*O*-alkyl) or D-Phe(*p*-alkyl) substitution in the OT antagonist series also led to enhanced anti-OT potency over their respective L-isomeric analogs in both *in vitro* and *in vivo* assays. The increase in anti-OT potency was particularly remarkable in the *in vitro* pA_2 assays for the 2-*O*-ethyltyrosine and the 2-*p*-ethylphenylalanine analogs, with increases as high as two orders of magnitude. The D-stereoisomer substitution, however, had no significant effect on the duration of *in vivo* inhibitory action. The recovery $t_{1/2}$ of the 2-D-isomer-containing analog was not significantly different statistically from its L-isomer-containing analog.

As we reported in our previous paper (2) and as shown in Table I, 8-ornithine substitution of [Pen^1]OT prolonged action but had little effect on potency, while 4-threonine substitution increased only antagonistic potency but not the duration of action. Sawyer *et al.* (14), however, reported that 8-ornithine as well as 4-threonine substitution enhances the antagonistic activity of [deamino-Pen^1]OT on the rat uterus. A combination of 2-*O*-alkyltyrosine or 2-*p*-alkylphenylalanine and 8-ornithine substitution produced the longest-acting OT antagonists in our series to date. It is interesting to note that increasing the size of the 2-*O*-alkyl (or 2-*p*-alkyl) substituent from a methyl group to an ethyl group resulted in a marked loss in antagonistic potency but a variable effect on the duration of action.

In this present study, we quantitated the anti-OT potency by *in vitro* pA_2 assays on nonpregnant rat uteri in a Mg^{2+}-free medium while the duration of action was measured in intact 21- or 22-day pregnant rats. Determination of *in vivo* pA_2's was not feasible for OT antagonists with very prolonged action. However, an estimate of relative *in vivo* anti-OT potency of the OT antagonists could be assessed since the *in vivo* recovery $t_{1/2}$'s were compared based on approximately equieffective doses of the OT antagonists which produced a 50–80% inhibition of the contractile response to the standard dose of OT. It is well known that potencies assayed *in vitro,* particularly in a Mg^{2+}-free medium, correlate poorly with *in vivo* potencies (14, 17). This is apparent here by comparing the *in vitro* pA_2's and the relative *in vivo* potencies in Table I. The disparity was particularly apparent with [Pen^1,Tyr(OEt)^2,Thr^4,Orn^8]OT. This OT antagonist has the lowest *in vitro* pA_2 value

among the OT antagonists studied in this paper. However, its *in vivo* anti-OT potency was unexpectedly high. Anti-OT activity and recovery $t_{1/2}$ of this OT antagonist were determined in five 21- or 22-day pregnant rats. All five experiments produced an unequivocal inhibition of OT responses with 8 μg of the antagonist in four rats and 6 μg in one rat. The OT dose was 50–80 mU. The inhibition was between 50 and 54% of the control OT response. In two other experiments, results not included in Table I, 30 μg of [Pen^1,Tyr-(OEt)^2,Thr^4,Orn^8]OT was found to produce a near complete inhibition of the OT response. It should be noted that the *in vivo* anti-OT assays were semiquantitative and were estimated from experiments designed to determine recovery $t_{1/2}$'s. The experimental protocol for the recovery $t_{1/2}$ determination allowed a response (inhibition) range of 50 to 80%. Therefore, the relative *in vivo* anti-OT potency range assessed from the recovery $t_{1/2}$ experiments could vary as much as four- to fivefold from one end of the response range to the other. For [Pen^1,Tyr(OEt)^2,Thr^4,Orn^8]OT, its relative *in vivo* anti-OT potency was probably overestimated because the assays were done in the low inhibitory response range. For this reason, we had placed this antagonist last within the intermediate potent group in the initial publication (2, Table 4).

Despite the lack of concordance between *in vitro* and *in vivo* potencies, the two sets of data on the effects of 2-D-amino acid substitution on the three OT antagonists yield similar conclusions, namely, potency and duration of action of OT antagonists could be dissociated.

The nonparallel effects of stereoisomer and amino acid substitutions on potency and duration of action observed in this study and our earlier studies are in accord with and support our hypothesis that potency and duration of action of these peptides may each have different molecular requirements (4, 5, 7, 8, 18).

The prolonged action of these OT antagonists cannot be explained by resistance to metabolic degradation or clearance since the long-lasting effect was also observed in the isolated rat uterus preparation. Deamino-OT, an agonist, was found to have a protracted action in our earlier study (17). Evidence was later presented by Gazis *et al.* (19) showing that the long-lasting action of deamino-OT is not due to a long plasma half-life or a greater binding affinity but is due to a specific binding of the peptide which remains in the uterine tissue. It is possible that the long-acting OT antagonists described here may also have a long retention in the uterine tissue. It is of particular interest to note that the time–action curves illustrated in Fig. 1 show that OT antagonists with a recovery $t_{1/2}$ of longer than 100 min produced their peak anti-OT effect during the 30- to 40-min interval following their injection. One explanation for this delayed peak action could be that the prolonged action resulted from favorable antagonist–receptor interactions that occurred *subsequent* to OT–receptor binding and transduction. Implicit in this latter suggestion is that the antagonist–receptor interaction has different conformational features than the agonist–receptor interaction as proposed in our hypothesis (3–5, 7, 8).

Although the exact mechanism remains to be elucidated, the findings reported here and in our earlier paper (2) give encouragement that OT analogs can be designed rationally based on conformational and structural considerations which are related to potency, specificity, and prolonged action, and that clinical deployment of OT antagonists as tocolytic agents for the treatment of premature labor may be realized with the development of further improved OT antagonists.

The authors acknowledge the skillful technical assistance of Ms. Lore von Hoffen and Ms. Marissa Putnam.

1. Chan WY, Hruby VJ. Oxytocin and prostaglandins in uterine contractions at term and the potential of oxytocin antagonists in the prevention of preterm labor. In: Hruby JV, Rich DH, Eds. Peptides, Structure and Function. Rockford, IL: Pierce Chemical Co, pp857–860, 1983.
2. Chan WY, Hruby VJ, Rockway TW, Hlavacek J. Design of oxytocin antagonists with prolonged action: Potential tocolytic agents for the treatment of preterm labor. J Pharmacol Exp Ther **239**:84–87, 1986.
3. Hruby VJ, Mosberg HI, Hadley ME, Chan WY, Powell AM. Synthesis, pharmacological, conformational, and dynamic studies of the potent hormone antagonists [1-penicillamine,4-threonine]oxytocin and [1-penicillamine, 2-phenylalanine, 4-threonine]oxytocin. Int J Peptide Protein Res **16**:372–381, 1980.
4. Hruby VJ, Mosberg HI, Sawyer TK, Knittel JJ, Rockway TW, Ormberg J, Darman P, Chan WY, Hadley ME. Conformational and dynamic consid-

erations in the design of peptide hormone analogs. Biopolymers **22**:517–530, 1983.
5. Hruby VJ, Knittel JJ, Mosberg HI, Rockway TW, Wilkes BC, Hadley ME. Conformational considerations in the design of highly potent and long-acting peptide hormone agonists and antagonists. In: Blaha K, Malon P, Eds. Peptides 1982. Berlin: de Gruyter, pp19–30, 1983.
6. Chan WY, Fear R, du Vigneaud V. Some pharmacologic studies on 1-L-penicillamine-oxytocin and 1-deaminopenicillamine-oxytocin: Two potent oxytocin inhibitors. Endocrinology **81**:1267–1277, 1967.
7. Hruby VJ. Conformational and dynamic considerations for the design of peptide superagonists and antagonists. In: Vida JA, Gordon M, Eds. Conformationally Directed Drug Design. Washington, DC: ACS Symposium Series, No. 251, pp9–27, 1984.
8. Hruby VJ, Hadley ME. Binding and information transfer in conformationally restricted peptides. In: van Binst G, Ed. Design and Synthesis of Organic Molecules Based on Molecular Recognition. Heidelberg: Springer-Verlag, pp269–289, 1986.
9. Schild HO. pA, a new scale for the measurement of drug antagonism. Brit J Pharmacol **2**:189–206, 1947.
10. Chan WY, Nestor JJ, Ferger MF, du Vigneaud V. Inhibition of oxytocic response to oxytocin in pregnant rats by [1-L-penicillamine]oxytocin and [1-β-mercapto-β-β-diethylpropionic acid]oxytocin. Proc Soc Exp Biol Med **146**:364–366, 1974.
11. Takahashi K, Diamond F, Bieniarg J, Yen H, Burd L. Uterine contractility and oxytocin sensitivity in preterm, term and post-term pregnancy. Amer J Obstet Gynecol **136**:774–779, 1980.
12. Fuchs AR. Oxytocin in animal parturition. In: Amico JA, Robinson AG, Eds. Oxytocin: Clinical and Laboratory Studies. Amsterdam: Elsevier, pp207–235, 1985.
13. Fuchs F. Role of maternal and fetal oxytocin in human parturition. In: Amico JA, Robinson AG, Eds. Oxytocin: Clinical and Laboratory Studies. Amsterdam: Elsevier, pp236–256, 1985.
14. Sawyer WH, Haldar J, Gazis D, Seto J, Bankowski K, Lowbridge J, Turan A, Manning M. The design of effective *in vivo* antagonists of rat uterus and milk ejection response to oxytocin. Endocrinology **106**:81–91, 1980.
15. Manning M, Sawyer WH. Design of potent and selective *in vivo* antagonists of the neurohypophysial peptides. In: Cross BA, Leng G, Eds. The Neurohypophysis: Structure, Function and Control. Progress in Brain Research. New York: Elsevier, Vol **60**:pp367–381, 1983.
16. Manning M, Olma A, Klis WA, Kolodziejczyk AM, Seto J, Sawyer WH. Design of more potent antagonists of the antidiuretic response to arginine vasopressin. J Med Chem **25**:45–50, 1982.
17. Chan WY, Kelly N. A pharmacologic analysis on the significance of the chemical functional groups of oxytocin to its oxytocic activity and on the effect of magnesium on the *in vitro* and *in vivo* oxytocic activity of neurohypophysial hormones. J Pharmacol Exp Ther **156**:150–158, 1967.
18. Hruby VJ. Structures of the neurohypophyseal hormones and analogs, and implications for hormone-receptor interactions. In: Litwack G, Ed. Biochemical Actions of Hormones. New York: Academic Press, pp191–241, 1986.
19. Gazis D, Roy J, Schwartz IL. Prolonged responses by the rat uterus *in vivo* to deamino-oxytocin disproportionate to its estimated plasma half-life. Endocrinology **106**:805–810, 1980.

Received October 8, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted February 25, 1987.