Effect of Threonine in Position 4 in Oxytocin and Vasotocin Analogs on the Time Course of Uterotonic Response

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The substitution of glutamine by threonine in position 4 of oxytocin, deamino-oxytocin, deamino-1-carba-oxytocin, and deamino-6--carba-oxytocin was found to increase the elimination rate of all analogs examined from the uterine receptor compartment in rat. However, this substitution was without any effect on the time course of uterotonic response in the case of vasotocin and deamino-vasotocin. These results suggest that the topological relationships of the 4 and 8 positions may show an important effect on elimination rate of oxytocin and vasotocin analogs from the rat uterine compartment.

Some single changes in the molecule of oxytocin or its analogs such as the substitution of glutamine in position 4 by threonine [Manning et al. 1970a, b], the substitution of cysteine in position 1 by mercaptopropionic acid [Hope et al. 1962] or the replacement of disulfide bridge by thioether bridging group [Jošt et al. 1973, Jošt 1987] (carba analogs) produced synthetic analogs with uterotonic activity (rat uterus) exceeding that of oxytocin several times. The combination of the last two substitutions resulting in deamino-carba-analogs [Barth et al. 1973, 1975] led to a further increase of the activity, while the introduction of threonine into the molecule of deamino-oxytocin or deamino-carba-oxytocin was accompanied by the decrease of uterotonic activity *in vitro* [Manning et al. 1971; Lebl et al. 1985]. 4-Threonine analogs of deamino-1-carba- and deamino-6-carba--oxytocin displayed also lower biological activity in rat uterotonic test *in vivo* than the analogs containing glutamine in position 4. Furthermore, we have noticed that the effect of 4-threonine-analogs not only fades away faster than that of deamino-carba-oxytocins, but also faster than the effect of oxytocin.

Since the substitution of cysteine by mercaptopropionic acid or carba substitution of the bridge not only increases the uterotonic potency of appropriate analogs, but also significantly decreases the fading of uterotonic response [Barth et al. 1974], we decided to study this effect using a series of oxytocin and vasotocin analogs with threonine in position 4 with special attention to the effect of these substitutions on the duration of this response.

Materials and Methods

In this study, the following analogs (Tab. 1) were used: [4-threonine]oxytocin [Manning et al. 1970a, b], [4-threonine] deamino-oxytocin [Manning et al. 1971], [4,-threonine]-deamino-1-carba-oxytocin [Lebl et al. 1985], [4--threonine]deamino-6-carba-oxytocin [Lebl et al. 1985], [8-arginine]vasotocin [Manning et al. 1973a], [4-threonine,8-arginine]vasotocin [Manning et al. 1973a], [8-arginine]deamino-vasotocin [Manning et al. 1973b] and

Table 1

The analogs used in this study

A-Tyr-Ile-B-Asn-C-Pro-D-Gly-NH_2

	A	B	C	D
Oxytocin	Cys	Gln	Cys	Leu
[4-Threonine]oxytocin	Cys	Thr	Cys	Leu
[4-Threonine]deamino-oxytocin	Mpr	Thr	Cys	Leu
[4-Threonine]deamino-1-carba-oxytocin	CH_2—CO CH_2—CH_2	Thr	Cys	Leu
[4-Threonine]deamino-6-carba-oxytocin	Mpr	Thr	NH—CH—CO CH_2—CH_2	Leu
[8-Arginine]vasotocin	Cys	Gln	Cys	Arg
[4-Threonine,8-arginine]--vasotocin	Cys	Thr	Cys	Arg
[8-Arginine]deamino-vasotocin	Mpr	Gln	Cys	Arg
[4-Threonine,8-arginine]--deamino-vasotocin	Mpr	Thr	Cys	Arg

[4-threonine,8-arginine]deamino-vasotocin [Manning et al. 1973b]. Female rats weighing 180—210 g were prepared for the experiment as described previously [Barth et al. 1974]. Estrogenized female rats were anesthetized with 15 % ethanol (total amount 6 % of body weight) administered by a stomach catheter. After laparotomy a ligature was passed around the uterine horn. The thread used for the ligature of the uterine horn was fastened to the arm of a magnetoelectric compensation transducer. The uterine contractions were measured isometrically. Oxytocin and its analogs were administered into the femoral vein. The formal elimination constants were calculated by following the rate of decline of the response using the two dose method [Řežábek and Kynčl 1966]. Oxytocin was used as a standard.

Results

When structural modifications of oxytocin (Fig. 1) which previously had provided some prolongation of the uterotonic response *in vivo* (see above) were used in conjunction with the substitution of glutamine in position 4 by threonine, the analogs did not display any protracted action in uterotonic test *in vivo* (Tab. 2). All four oxytocin analogs containing threonine in position 4 showed basically the same elimination constant. This fact suggests that the properties of the molecule were significantly changed by the introduction of threonine in place of glutamine, so that the

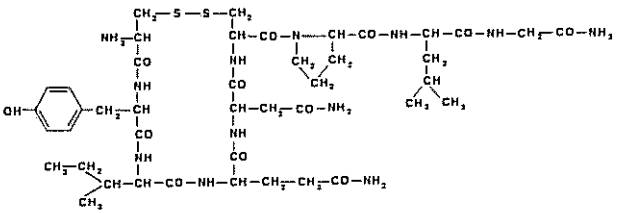

Fig. 1. Structural formula of oxytocin.

structural features normally leading to some prolongation of the activity (i. e. the absence of primary amino group or carba-substitution of the disulphide bridge) no longer provided analogs with enhanced duration of the uterotonic effect. This change of the activity of 4-threonine analogs is limited to oxytocin analogs, since similarly substituted vasotocin analogs (Tab. 3) show the expected prolongation of the activity by deamination in position 1. The substitution of leucine in position 8 by arginine resulted in a decrease of elimination rate from uterine receptor compartment (compare the formal elimination constants of [8-arginine]vasotocin and [4--threonine,8-arginine]vasotocin with those of oxytocin and [4-threonine]oxytocin). The substitution of cysteine by 3-mercaptopropionic acid in position 1 resulted in a decrease of elimination constant in oxytocin as well as in [8-arginine]vasotocin. However, there was a dramatic differ-

ence in the effect of substitution by threonine in position 4. Thus, vasotocin and deamino-vasotocin analogs containing threonine showed basically the same formal elimination constant as the corresponding analogs with glutamine, contrary to the oxytocin analogs, where threonine in position 4 eliminated the prolongation of the activity brought about by the deamination. The only difference between these two groups of compounds was the basic arginine residue and lipophilic leucine residue in position 8 of vasotocin and oxytocin, respectively.

Table 2

Formal elimination constants of oxytocin analogs from the rat uterine compartment

Compound	k, min^{-1}
Oxytocin	0.250 ± 0.070[1]
[4-Threonine]oxytocin	0.333 ± 0.041 n = 3
Deamino-oxytocin	0.148 ± 0.044[1]
[4-Threonine]deamino-oxytocin	0.323 ± 0.078 n = 4
Deamino-1-carba-oxytocin	0.172 ± 0.050[1]
[4-Threonine]deamino-1-carba-oxytocin	0.320 ± 0.075 n = 5
Deamino-6-carba-oxytocin	0.041 ± 0.011[1]
[4-Threonine]deamino-6-carba-oxytocin	0.353 ± 0.058 n = 6

[1] Taken from Barth et al. [1974].

Table 3

Formal elimination constants of vasotocin analogs from the rat uterine compartment

Compound	k, min^{-1}
[8-Arginine]vasotocin	0.190 ± 0.014 n = 11
[4-Threonine,8-arginine]vasotocin	0.206 ± 0.039 n = 8
[8-Arginine]deamino-vasotocin	0.094 ± 0.013 n = 6
[4-Threonine,8-arginine]deamino-vasotocin	0.097 ± 0.016 n = 11

Discussion

The substitution of glutamine by threonine in position 4 was found to increase the potency of oxytocin analogs [Manning et al. 1970 a, b] significantly more than in the case of vasotocin or vasopressin analogs [Manning et al. 1973a, b]. The deamination which does not increase but rather decreases the activity of 4-threonine analogs of oxytocin showed a less pronounced influence on the uterotonic activity of vasotocin and vasopressin analogs [Manning et al. 1973a, b]. The non-cooperativity of deamination and 4-threonine substitution in the enhancement of activity of oxytocine analogs was explained by an improper hydrophobic-hydrophilic balance of the resulting analogs [Manning et al. 1971] (or their too high lipophilicity). The substitution by arginine in position 8 makes the analog more hydrophilic and therefore the deamination in position 1 does not make it excessively lipophilic. This might be the reason for the less pronounced influence of deamination on the biological activity of vasotocin analogs. However, the term "local lipophilicity" may be more appropriate here, since a long list of analogs with higher overall lipophilicity but still retaining high potency are known [for review see Lebl et al. 1987]. On the other hand, the biological activity of [4-threonine,7-glycine]oxytocin [Lowbridge et al. 1977; Stahl and Walter 1977] supports our idea of lipophilicity compensation, since this analog was shown to have protracted activity *in vitro* [Stahl and Walter 1977].

Prolongation of the biological activity might result from intercompartmental transport of the analog in the living organism [Vaněčková et al. 1976; Gazis et al. 1980; Smith 1981]. Highly lipophilic analog might be unavailable for this transport due to a strong interaction in one of the compartments which might result in the loss of prolongation if this is retained in the "wrong" compartment. An alternative explanation that conformational structure features are related to peptide hormone-receptor interactions in the post-transduction state (the pi state) has been suggested [Hruby et al. 1983, 1984; Hruby and Handley 1986] to explain the prolongation of action in oxytocin and other peptide hormones and neurotransmitters. Implicit in this explanation is the possibility for increasing or decreasing the population of a particular hormone-receptor bound state. In this case the prolongation can result from a receptor-ligand interaction involving a few residues or some region of the peptide, but in conjunction with other topological features of the peptide-receptor interaction than those involved in binding (potency) or transduction (efficacy). The results presented here are consistent with this hypothesis, and, in fact, suggest that the topographical relationships of the position 4 and 8 side chains are critical for prolonged activity, at least as far as the target tissue--uterus is concerned.

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