COMPARISON OF BINDING AFFINITIES OF A SERIES OF OXYTOCIN ANALOGUES TO UTERINE AND MAMMARY GLAND RECEPTORS AND THEIR BIOLOGICAL POTENCIES

Jiřina SLANINOVÁ[a], Mario HACKENBERG[b] and Falk FAHRENHOLZ[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia*

[b] *Max-Planck-Institute of Biophysics, Kennedy Allee 70, Frankfurt a.M. 70, F.R.G.*

Received June 27, 1990
Accepted July 30, 1990

The affinity of 12 oxytocin analogues of similar structure but differences in conformational freedom and agonistic and antagonistic properties to receptors in guinea pig uterus and rat mammary gland membrane preparations was determined by competitive binding experiments using tritiated oxytocin. The values obtained for the apparent dissociation constant K_D were compared to the values of biological activities from classical pharmacological tests (uterotonic test in vitro and galactogogic test in vivo).

Theoretically, the affinity constants of analogues for their receptors obtained from binding studies should fit the affinities calculated from biological activity determinations better in the case of competitive antagonists than in the case of agonists and better in the case of in vitro tests than in in vivo tests, where the values of effective concentrations are only arbitrarily calculated. For elucidation of response differences to neurohypophyseal hormone analogues of two tissues (smooth muscle, myoepithelial cell) for which the same mechanism of action is proposed[1], the receptor binding studies can still provide valuable information.

The analogues studied in this work differ mainly in the conformational flexibility in certain parts of the molecule which gives rise to different biological activities. We wondered how this changes affect affinities of these analogues for the receptors as determined in binding studies.

EXPERIMENTAL

Materials. [L-Phe[2]]oxytocin*, [D-Phe[2]]oxytocin, [L-Tic[2]]oxytocin, [D-Tic[2]]oxytocin, [L-*o*-

* Unless stated otherwise, all chiral amino acids belong to the L-series. Nomenclature and symbols of the amino acids and peptides obey the published IUPAC recommendations[2]. Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; *o*-MePhe, 2′-methylphenylalanine; Cpp,3,3-cyclopentamethylene-3-mercaptopropionic acid.

-MePhe^2]oxytocin, [D-*o*-MePhe^2]oxytocin and deaminooxytocin were synthesized in the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences and had qualities as described in refs[3,4]. The [Glu^4,Lys^8]deaminooxytocin and [Lys^4,Glu^5]deaminooxytocin and their bicyclic analogues are described in refs[5,6]. Oxytocin was from Bachem, [^{3}H]oxytocin from NEN.

Methods. Membranes were prepared either from guinea pig uteri of 60th day of pregnancy or from rat mammary glands 26 days after parturition (6 days after weaning), according to the methods described in refs[7,8] with minor modifications. A crude membrane fraction was used. The binding tests were performed as described in ref.[7]. The analogues were tested in the concentration range $1 \cdot 10^{-11}$—$3 \cdot 10^{-6}$ mol/l. The K_D values were obtained using a weighted non-linear least squares fit to logistic curves[9].

TABLE I

Binding characteristics and biological activities of some oxytocin analogues

	Analogue	K_D (mol/l)		Activity, IU/mg or pA_2	
		uterus	mammary gland	uterus in vitro	galactogogic test in vivo
I	OXT	$1{\cdot}7 \cdot 10^{-9}$	$9{\cdot}4 \cdot 10^{-10}$	450	450
II	[Mpa^1]OXT	$1{\cdot}1 \cdot 10^{-9}$	$1{\cdot}3 \cdot 10^{-9}$	803	541
III	[L-Phe^2]OXT	$7{\cdot}9 \cdot 10^{-10}$	$4{\cdot}4 \cdot 10^{-9}$	22·2	175
IV	[D-Phe^2]OXT	$1{\cdot}5 \cdot 10^{-7}$	$7{\cdot}5 \cdot 10^{-7}$	pA_2 = 7·4 ($4 \cdot 10^{-8}$)	1·2
V	[L-Tic^2]OXT	$>10^{-6}$	10^{-6}	pA_2 = 5·6 ($2{\cdot}5 \cdot 10^{-6}$)	0·01
VI	[D-Tic^2]OXT	$>10^{-6}$	$7{\cdot}3 \cdot 10^{-7}$	pA_2 = 6·7 ($2 \cdot 10^{-7}$)	0·17
VII	[L-*o*-MePhe^2]OXT	$3{\cdot}5 \cdot 10^{-8}$	$7{\cdot}1 \cdot 10^{-8}$	pA_2 = 6·7 ($2 \cdot 10^{-7}$)	2·35
VIII	[D-*o*-MePhe^2]OXT	$1{\cdot}8 \cdot 10^{-8}$	$3{\cdot}7 \cdot 10^{-8}$	pA_2 = 7·6 ($2{\cdot}5 \cdot 10^{-8}$)	>0·05
IX	[Mpa^1,Glu^4,Lys^8]OXT	$2{\cdot}2 \cdot 10^{-7}$	$6{\cdot}0 \cdot 10^{-7}$	0·4	9·1
X	[Mpa^1,Glu^4,Lys^8, 4→8 cyclo]OXT	$1{\cdot}1 \cdot 10^{-7}$	$2{\cdot}3 \cdot 10^{-7}$	pA_2 = 8·2 ($6{\cdot}4 \cdot 10^{-9}$)	mixed character
XI	[Mpa^1,Lys^4,Glu^5]OXT	$>10^{-6}$	$>10^{-6}$	0	0
XII	[Mpa^1,Lys^4,Glu^5, 4→5 cyclo]OXT	$>10^{-6}$	$>10^{-6}$	0	0

RESULTS AND DISCUSSION

The K_D values of the analogues studied are summarized in Table I, together with the data of the biological activities. Figure 1 illustrates the binding curves obtained. The binding of [^{3}H]oxytocin to both membrane preparations was dose and time dependent and saturable. The Scatchard analysis of the binding resulted in the K_D values of $(1{\cdot}22 \mp 0{\cdot}02) \cdot 10^{-9}$ mol/l and $(1{\cdot}33 \mp 0{\cdot}03) \cdot 10^{-9}$ mol/l for uteri and mammary gland membranes, respectively. As can be seen from the data, restriction of the conformational freedom resulted in all cases in decrease of the affinity of the analogues for the receptors.

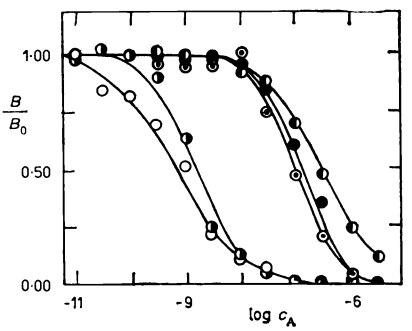
TABLE II

Binding and activity ratios of analogues determined in test on mammary gland

Analogue	K_D analogue/K_D OXT	Activity OXT/Activity analogue
I	1	1
II	1·38	0·83
III	4·68	2·57
IV	797	375
V	>1 000	45 000
VI	776	2 647
VII	75·5	191
VIII	39	>9 000
IX	638	49
X	244	—
XI	>600	>1 000
XII	>600	>1 000

FIG. 1

An example of binding curves obtained under described conditions using uterine membranes. *B* means specific binding in the presence of a given concentration (c_A) of oxytocin or the analogue, B_0 means specific binding in the absence of unlabelled peptide, ○ Oxytocin, ◑ [L-Phe^2]oxytocin, ● [D-Phe^2]-oxytocin, ◐ [Mpa^1,Glu^4,Lys^8]oxytocin, ⊙ [Mpa^1, Glu^4, Lys^8, 4→8 cyclo]oxytocin



The same series of analogues was tested for binding at both kinds of membranes. The K_D values obtained from binding experiments and the data of biological activity expressed either in IU/mg or pA_2 were compared. The pA_2 value has an exact interpretation in the in vitro experiment, it corresponds to the negative decadic logarithm of the dissociation constant of the antagonist–receptor complex. This value should be directly comparable to the K_D values. However, for the uterus system we found no correlation between the K_D values and the pA_2 values. The most striking was the discrepancy in the case of the very potent oxytocin antagonist (*X*) which on the one hand showed a low binding affinity (65-fold lower than oxytocin), on the other hand was a potent antagonist. The discrepancies may be due to yet unexplored differences in receptor qualities (mechanism of signal transfer, interplay of vasopressin and oxytocin receptors) and/or to ligand specificities of receptors from different species (guinea pig versus rat). There are also opposite cases described, i.e. analogues which have very high affinities for the uteri receptors however their antagonistic potency is not correspondingly high (e.g.[Cpp^1,Tyr(Me)^2,Thr^4,Orn^8,Tyr^9]-oxytocin, K_D = 0·17 nmol/l versus pA_2 = 7·83 (ref.[10])). All these phenomena should be further studied.

A different result was obtained for the mammary gland system where both experiments – binding and biological evaluation – were done with the same species and all the compounds studied displayed agonistic activity (with the exception of substance *X* which showed a mixed character). A weak correlation can be found if we compare the receptor affinity with the biological activity of the analogues relative to oxytocin (Table II). Our findings are more or less in accordance with literature[11] and point out that making conclusions from binding data about biological potencies may be rather misleading.

This work was partly supported by DAD *Society (J.S.). We would like to thank to Drs V. J. Hruby, M. Lebl and P. Majer for yielding the analogues and to Mrs I. Hošková for perfect technical assistance.*

REFERENCES

1. Gazis D. in: *Handbook of Neurohypophyseal Hormone Analogs* (K. Jošt, M. Lebl and F. Brtník, Eds) Vol. I, Part. 2, p. 51. CRC Press, Boca Raton 1987.
2. Eur. J. Biochem. *138*, 9 (1984).
3. Lebl M., Hill P., Kazmierski W., Karaszova L., Slaninová J., Frič I., Hruby V. J.: Int. J. Pept. Protein Res. *36*, 321 (1990).
4. P. Majer, Slaninová J., Lebl M.: Unpublished results.
5. Hill P. S., Slaninová J., Hruby V. J.: Unpublished results.
6. Hill P. S., Smith D. D., Slaninová J., Hruby V. J.: J. Am. Chem. Soc. *112*, 3110 (1990).
7. Soloff M. F., Fernstrom M. A.: Endocrinology *120*, 2474 (1987).
8. Fahrenholz F., Hackenberg M., Muller M.: Eur. J. Biochem. *174*, 81 (1988).
9. Fahrenholz F., Boer R., Crause P., Toth M. V.: Eur. J. Biochem. *152*, 589 (1985).

10. Elands J., Barberis C., Jard S., Tribollet E., Dreifuss J. J., Bankowski K., Manning M., Sawyer W. H.: Eur. J. Pharmacol. *147*, 197 (1988).
11. Atke A., Vilhardt H., Melin P.: J. Endocrinol. *118*, 187 (1988).

Translated by the author (J.S.).