Acta Biochimica Polonica

Vol. 54 No. 4/2007, 805–811

on-line at: www.actabp.pl

Regular paper

Oxytocin analogues with amide groups substituted by tetrazole groups in position 4, 5 or 9

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Received: 11 September, 2007; revised: 26 November, 2007; accepted: 04 December, 2007
available on-line: 17 December, 2007

Eleven oxytocin analogues substituted in position 4, 5 or 9 by tetrazole analogues of amino acids were prepared using solid-phase peptide synthesis method and tested for rat uterotonic *in vitro* and pressor activities, as well as for their affinity to human oxytocin receptor. The tetrazolic group has been used as a bioisosteric substitution of carboxylic, ester or amide groups in structure–activity relationship studies of biologically active compounds. Replacement of the amide groups of Gln^4 and Asn^5 in oxytocin by tetrazole analogues of aspartic, glutamic and α-aminoadipic acids containing the tetrazole moiety in the side chains leads to analogues with decreased biological activities. Oxytocin analogues in which the glycine amide residue in position 9 was substituted by tetrazole analogues of glycine had diminished activities as well. The analysis of differences in rat uterotonic activity and in the affinity to human oxytocin receptors of analogues containing either an acidic 5-substituted tetrazolic group or a neutral 1,5- or 2,5-tetrazole nucleus makes it possible to draw some new conclusions concerning the role of the amide group of amino acids in positions 4, 5 and 9 of oxytocin for its activity. The data suggest that the interaction of the side chain of Gln^4 with the oxytocin receptor is influenced mainly by electronic effects and the hydrogen bonding capacity of the amide group. Steric effects of the side chain are minor. Substitution of Asn^5 by its tetrazole derivative gave an analogue of very low activity. The result suggests that in the interaction between the amide group of Asn^5 and the binding sites of oxytocic receptor hydrogen bonds are of less importance than the spatial requirements for this group.

Keywords: oxytocin analogues, SAR, tetrazole derivatives

INTRODUCTION

Oxytocin (OT, Fig. 1), a neurohypophyseal hormone, stimulates contraction of the uterine myometrium at parturition and contracts myoepithelium cells during lactation. This hormone acts through specific oxytocin receptors (OTR), which belong to the Class A G-protein-coupled receptor family (GPCR). Results of structure–activity relationship studies of OT showed that the amide groups of glutamine, asparagine and glycine residues in positions 4, 5 and 9, respectively, play important but distinct roles for the activity of OT and its interaction with OTR (Manning *et al.*, 1981; Manning &

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Abbreviations: Aad(T), 2-amino-5-(5-tetrazolyl)pentanoic acid, *δ-tetrazole analogue of α-aminoadipic acid*; Asp(T), 2-amino-3-(5-tetrazolyl)propionic acid, *β-tetrazole analogue of aspartic acid*; Asp(T^{1}CH$_3$), 2-amino-3-[5-(2-methyl)tetrazolyl]propionic acid; Asp(T^{2}CH$_3$), 2-amino-3-[5-(3-methyl)tetrazolyl]propionic acid; DIPEA, *N,N*-diisopropylethylamine; Boc, *t*-butoxycarbonyl; DMF, *N,N*-dimethylformamide, Fmoc, 9-fluorenylmethoxycarbonyl; Glu(T), 2-amino-4-(5-tetrazolyl)butanoic acid, *γ-tetrazole analogue of glutamic acid*; Glu(T^{1}CH$_3$), 2-amino-4-[5-(2-methyl)tetrazolyl]butanoic acid; Glu(T^{2}CH$_3$), 2-amino-4-[5-(3-methyl)tetrazolyl]butanoic acid; GlyT, 5-aminomethyltetrazole, *α-tetrazole analogue of glycine*; GlyT^{1}CH$_3$, 5-aminomethyl-2-methyltetrazole; GPCR, G-protein coupled receptor; OT, oxytocin; OTR, oxytocin receptor; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIPS, triisopropylsilane; Z, benzyloxycarbonyl.

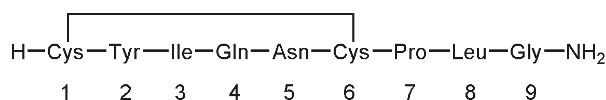

Figure 1. Structure of oxytocin (OT).

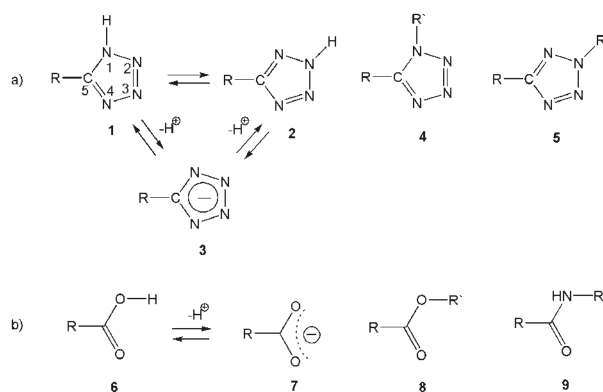

Figure 2. Structural relations between 5-substituted tetrazoles (a) and carboxylic acids, esters and amides (b).

Sawyer, 1985; Lebl *et al.*, 1987; Tarnowska *et al.*, 1992; Gimpl & Fahrenholz, 2001; Ślusarz *et al.*, 2006). The glutamine amide of OT can be replaced by various groups without a pronounced drop of uterotonic activity. Substitution of Gln by Thr gave an analogue with an almost doubled uterotonic activity (Manning *et al.*, 1970). On the other hand, asparagine in position 5 is the most crucial residue for OT activity. Removal or substitution of the amide group of Asn led to analogues with very diminished activity. The most active analogue was [Asp^5]OT, which retained 4% uterotonic activity of OT (Walter *et al.*, 1978). Most modifications of the glycine amide in position 9 led to a considerably reduced uterotonic activity, with the exception of the 9-aminoacetonitrile analogue which was even more active than the parent hormone (Roy *et al.*, 1983).

We present here the synthesis and biological activities of 11 oxytocin analogues, in which the carboxamide group in positions 4, 5 or 9 was substituted by the bioisosteric tetrazolyl group (Butler, 1984; Burger, 1991; Herr, 2002). 5-Substituted tetrazoles, frequently referred to as tetrazolic acids, behave as acids comparable in character to carboxylic acids. Their pK_a values are in the same range as those of carboxylic acids (Herbst & Wilson, 1957; Kaczmarek, *et al.*, 1979). Therefore, they are ionized at a physiological pH. 5-Substituted tetrazoles exist as 1*H*- and 2*H*-tautomers (Fig. 2). Tetrazolic acids and especially tetrazolate anions have high capacity for hydrogen bond formation in the interaction with other molecules (Rażyńska *et al.*, 1983). Hansch and Leo reported that tetrazolate ions are almost 10 times more lipophilic than the corresponding carboxylic anions (1995). This property and the high metabolic stability of tetrazolyl group are important factors for the design of potential drugs. Considering the structure of 5-substituted tetrazoles and their N^1- or N^2-substituted derivatives, they can mimic esters and amides as well (Fig. 2). Therefore, tetrazoles can be used in order to elucidate the role of hydrogen bonds formed by amide groups in the interaction of biologically active peptides with their receptors.

The aim of our study was to use the bioisosteric tetrazolyl group in the hope of increasing our understanding of the role of amide groups of OT for the biological functions of the hormone. Therefore, we have substituted the Gln^4, Asn^5 and Gly-NH$_2^9$ residues in OT by their tetrazole derivatives (Fig. 3) and studied biological properties of the resulting analogues.

EXPERIMENTAL PROCEDURES

Materials. Fmoc-amino acids and CLEAR-resin were purchased from Peptides International, Inc. Syntheses of Fmoc-derivatives of tetrazole analogues of glycine, α-aminoadipic (racemic or L-enantiomer), aspartic and glutamic acids have been reported recently (Manturewicz *et al.*, 2007; Manturewicz & Grzonka, 2007). TLC analysis was carried out on aluminium sheets precoated with silica gel 60, F-254 (Merck). RP-HPLC used for purification of products and for their analyses was performed on Kromasil C4 or C8 columns. ^{1}H-NMR spectra were measured on a Varian Mercury 400 MHz spectrometer. IR spectra were measured on a Bruker IFS66 spectrophotometer. Mo-

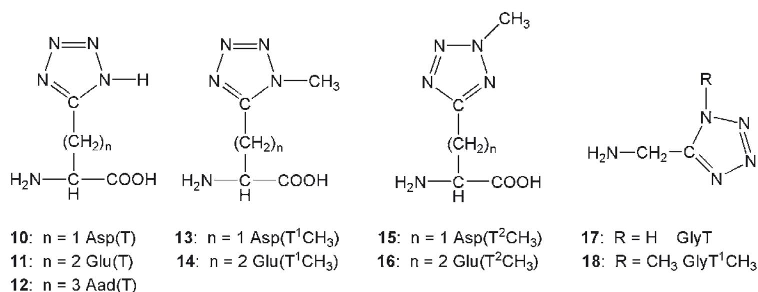

Figure 3. Tetrazoles analogues of amino acids in this study.

lecular weights of the compounds were determined on a Bruker BIFLEX III MALDI-TOF spectrometer.

Peptide synthesis. Oxytocin analogues were synthesized manually by solid-phase method on CLEAR-amide resin. [1–7]OT was synthesized on 2-chlorotrityl-resin. The following side chain protecting groups were used: *tert*-butyl for Tyr and trityl for Asn, Gln and Cys. Deprotection of Fmoc group was carried out using 20% piperidine/DMF (1 × 5 min, 1 × 20 min). The resin was washed subsequently 3 times with DMF, 3 times with dichloromethane, and once with DMF. Deprotection and couplings were monitored using the Kaiser ninhydrin test (Kaiser *et al.*, 1970). Coupling was performed in DMF with 3 equiv. of Fmoc-amino acids and 3 equiv. of TBTU or DIPEA. Coupling time was 2 h. In the case of Fmoc-Asp(T^2CH_3)-OH, Fmoc-Glu(T^1CH_3)-OH and Fmoc-L-Aaa(T)-OH, due to small amounts of these derivatives available, couplings were performed with 1 equiv. for 6 h. Free amino groups which were not acylated during couplings were blocked with the use of *N*-acetylimidazole. After completion of the synthesis, the peptidyl-resin was washed stepwise with DMF, methanol and ethyl ether and dried. The peptide was cleaved from the resin using a cocktail of TFA, TIPS, phenol and water (8.8 mL: 0.2 mL: 0.5 mL: 0.5 mL per g of the resin, respectively) for 2 h at room temp. under argon atmosphere with agitation. The cleaved resin was then filtered off, and the resultant solution was concentrated *in vacuo*. The crude oxytocin analogue was precipitated with cold ethyl ether. The ether layer was decanted after 15 min of centrifugation. This process of adding ether, centrifugation and decanting was repeated 5 times and the product was dried by stream of argon. The peptide was oxidatively cyclized with 0.1 M I_2 in MeOH using the standard procedure (Atherton & Sheppard, 1989). Then Dowex 1 × 8 (CH_3COO^- form) was added to the solution under stirring. After 20 min, the supernatant was removed, the solvent evaporated under reduced pressure, and the residue dissolved in water and lyophilized. The crude product was purified by RP-HPLC. When racemic Fmoc-Aad(T)-OH was used in the synthesis of appropriate oxytocin analogue, two diastereoisomeric peptides were obtained, which were separated by RP-HPLC. The separated [Aad(T)^4]OT was found to be identical with the oxytocin analogue synthesized from the L-enantiomer of Fmoc-Aad(T)-OH. The purity and identity of each oxytocin analogue was determined by analytical RP-HPLC and MALDI-TOF mass spectrometry.

Oxytocin analogues containing tetrazole analogues of glycine were synthesized by [1–7] and [8–9] fragment condensation. The appropriate analogues of dipeptide H-Leu-Aaa were synthesized as follows:

H-Leu-GlyT. Fmoc-Leu-OH (2 mmoles), 1-hydroxybenzotriazole (2 mmoles) and dicyclohexylcarbodiimide (2 mmoles) were added to the solution of H-GlyT (2 mmoles) in DMF (25 mL). The mixture was stirred for 5 h at –20ºC and the precipitated dicyclohexylurea was filtered off and washed with ethyl acetate. The solvents were evaporated, and the residue was dissolved in ethyl acetate. The organic layer was washed subsequently with 1 M HCl and brine and then dried over $MgSO_4$. Evaporation of the solvent gave 1.46 mmole (73% yield) of Fmoc-Leu-GlyT [MS(MALDI-TOF): for $C_{23}H_{26}N_6O_3$ – calc. 434.2; found 435.2 (M+H); 457.2 (M+Na); 473.2 (M+K)]. Fmoc-Leu-GlyT (1 mM) was treated with 20% piperidine in DMF for 15 min at room temp. The solution was alkalized with aqueous ammonia and washed with light petroleum, ethyl ether and ethyl acetate. Final product was obtained after lyophilization in 71% yield. MS [(MALDI-TOF)]: for $C_8H_{16}N_6O$ — calc. 212.1; found 213.2 (M+H).

H-Leu-GlyT^1*CH*$_3$. Z-Gly-OH (10 mmoles), 1-hydroxybenzotriazole (10 mmoles) and CH_3NH_2. HCl (10 mmoles) were dissolved in CH_2Cl_2 (50 mL) and DMF (4 mL). The solution was cooled to –20ºC and triethylamine (10 mM) and *N*-ethyl-*N'*-(3-dim-ethylaminopropyl)carbodiimide hydrochloride (10 mM) was added during 1 h with stirring at –20ºC. The reaction mixture was left overnight at room temp. The solvents were evaporated and the residue was dissolved in ethyl acetate. The organic layer was washed stepwise with water, 1 M $NaHCO_3$ and brine and then dried over $MgSO_4$. Evaporation of the solvent gave Z-Gly-$NHCH_3$. Yield 86%; m.p. 134–136ºC. Analysis: for $C_{11}H_{14}N_2O_3$ — calc. C 59.45%; H 6.35%; N 12.60%; found C 59.61%; H 6.44%; N 12.60%.

Quinoline (20 mmoles) was added to a cooled solution (10ºC) of PCl_5 (10 mmoles) in anhydrous chloroform (35 mL), and the solution was stirred for 15 min. Next Z-Gly-$NHCH_3$ (10 mmoles) was added in portions during 10 min. The reaction mixture was stirred for 30 min and then HN_3 in benzene (25 mmoles) was added. After 12 h, solvents were evaporated, and the residue was dissolved in ethyl acetate and washed subsequently with water, 1 M $NaHCO_3$, and brine and then dried over $MgSO_4$. The obtained Z-GlyT^1CH_3 was purified on a silica gel column with ethyl acetate/light petroleum (1:1, v/v) as an eluent. Yield: 36% (oil). Analysis: for $C_{11}H_{13}N_5O_2$ — calc. C 53.43%; H 5.30%; N 28.32%; found C 53.55%; H 5.41%; N 28.25%.

Z-GlyT^1CH_3 (1 mmole) was treated with HBr in acetic acid (1 mL) for 40 min followed by addition of dry ethyl ether (20 mL). The ethyl ether layer was decanted and the product was triturated with dry ether. HBr · H-GlyT^1CH_3 was dissolved in 1 M $NaHCO_3$ and extracted 3 times with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. Oily H-GlyT^1CH_3 was ob-

tained in 90% yield. [MS (MALDSI-TOF): for $C_3H_7N_5$ — calc. 113.1; found 114 (M+H), 136 (M+Na); 151.9 (M+K)].

Boc-Leu-GlyT^1CH_3 was obtained in 75% yield from Boc-Leu-OH and H-GlyT^1CH_3 in the same way as for Fmoc-Leu-GlyT. MS (MALDI-TOF): for $C_{14}H_{28}N_6O_3$ — calc. 326.2; found 327.3 (M+H); 348.2 (M+Na).

Deprotection of Boc-Leu-GlyT^1CH_3 was carried out using 80% trifluoroacetic acid in CH_2Cl_2 for 2 h at room temp. Solvents were evaporated and the residue was dissolved in ethyl acetate, washed with 1 M $NaHCO_3$, dried over $MgSO_4$ and evaporated. H-Leu-GlyT^1CH_3 was obtained in 96% yield. MS (MALDI-TOF): for $C_9H_{18}N_6O$ – calc. 226.2; found 227.2 (M+H).

Biological evaluation. Wistar rats were used in all experiments. Synthetic oxytocin and arginine-vasopressin were used as standards in the uterotonic test and pressor test, respectively. The uterotonic activity was determined *in vitro* on an isolated strip of rat uterus in the absence or presence of magnesium ions (Holton, 1948; Rudinger & Krejči, 1962). In principle, cumulative dosing was applied in most experiments, i.e. doses of standard or of the analogue were added successively to the uterus in the organ bath in doubling concentrations and at 1 min intervals without the fluid being changed until the maximal response was obtained. Each analogue was tested using uteri from 3–5 different rats. Pressor activity was determined on phenoxybenzamine-treated male rats in urethane anesthesia (Dekansky, 1952). The responses to standard doses of oxytocin or vasopressin were stable for several hours, without problems with tachyphylaxis. For more details concerning the tests see Slaninova (1987).

Binding affinity determination. Determination of binding affinity to human oxytocin receptor (OTR) was performed as described by Fahrenholz *et al*. (1984) using tritiated oxytocin from NEN Life Science (Boston, MA, USA). In brief, a crude membrane fraction of HEK OTR cells, i.e. HEK cells with stable expression of human OT receptors, kindly donated by Dr. G. Gimpl (Gimpl *et al*. 1997), was incubated with $[^3H]$OT (2 nM) and various concentrations of peptides (0.1–10 000 nM) for 30 min at 35°C. The total volume of the reaction mixture was 0.25 mL. Buffer used was 50 mM Hepes at pH 7.6 containing 10 mM $MnCl_2$ and 1 mg/mL bovine serum albumin. The reaction was terminated by quick filtration on a Brandel cell harvester. Binding affinities were expressed as IC_{50} values calculated from the binding curves using GraphPad Prism 3.02.

RESULTS AND DISCUSSION

Recently we have worked out methods of synthesis of Fmoc derivatives of tetrazole derivatives of Asp, Glu and Aad, compounds suitable for solid-phase peptide synthesis (Manturewicz & Grzonka, 2007b). Now, using these compounds we present manual solid-phase peptide synthesis of eleven new analogues of OT (**I–XI**) (Table 1) on a CLEAR-amide or 2-chlorotrityl resin following Fmoc chemistry (Atherton & Steward, 1989). All the analogues contain tetrazole derivatives of amino acids in which the tetrazole ring is either in the side chain

Table 1. Physicochemical properties of tetrazole analogues of oxytocin

	Analogue	HPLC[a] R_T (min)	Formula	[M+H+] Calculated	[M+H+] Found
I	$[Asp(T)^4]$OT	23.9	$C_{42}H_{63}N_{15}O_{11}S_2$	1018.4	1018.3
II	$[Asp(T^1CH_3)^4]$OT	11.2	$C_{43}H_{65}N_{15}O_{11}S_2$	1032.4	1032.4
III	$[Asp(T^2CH_3)^4]$OT	11.6	$C_{43}H_{65}N_{15}O_{11}S_2$	1032.4	1032.4
IV	$[Asp(T)^5]$OT	24.4	$C_{44}H_{66}N_{14}O_{11}S_2$	1031.4	1031.4
V	$[Glu(T)^4]$OT	24.3	$C_{43}H_{65}N_{15}O_{11}S_2$	1032.4	1032.3
VI	$[Glu(T^1CH_3)^4]$OT	11.2	$C_{44}H_{67}N_{15}O_{11}S_2$	1046.5	1046.4
VII	$[Glu(T^2CH_3)^4]$OT	11.8	$C_{44}H_{67}N_{15}O_{11}S_2$	1046.5	1046.4
VIII	$[Aad(T)^4]$OT	24.3	$C_{44}H_{67}N_{15}O_{11}S_2$	1046.5	1046.5
IX	$[D\text{-}Aad(T)^4]$OT	24.3	$C_{44}H_{67}N_{15}O_{11}S_2$	1046.5	1046.5
X	$[GlyT^9]$OT	17.4	$C_{43}H_{65}N_{15}O_{11}S_2$	1032.4	1032.3
XI	$[(GlyT^1CH_3)^9]$	29.0	$C_{44}H_{67}N_{15}O_{11}S_2$	1046.5	1046.5

[a]HPLC analyses were carried out on a Varian chromatograph equipped with a UV detector (λ = 223 nm). The following solvent systems were used: [A] 0.1% aqueous trifluoroacetic acid (TFA), [B] acetonitrile/0.1% aqueous TFA (80 : 20, v/v). The flow rate was 1 ml / min. Analyses of analogues **I**, **IV**, **V**, **VIII**, **IX**, **X**, **XI**, **XII** were carried out on a Kromasil C8 column (4.6 × 250 mm; 5 μm, 100 Å) using linear gradient elution from 2% to 100% of [B] in 60 min. Analyses of analogues **II**, **III**, **VI**, **VII** were carried out on a Kromasil C4 column (4.6 × 250 mm; 5 μm, 100 Å) using linear gradient elution from 0% to 100% of [B] in 30 min.

of appropriate amino-acid residues (derivatives of aspartic, glutamic or α-aminoadipic acids) or in the C-terminal position. The amide groups of glutamine in position 4 (analogues **I**, **V**, **VIII** and **IX**), asparagine in position 5 (analogue **IV**) or glycine amide in position 9 (analogue **X**) were substituted with acidic 5-tetrazolyl group. Other analogues (**II**, **III**, **VI**, **VII** and **XI**) contain N-methylated tetrazole ring (at N^1 or N^2 atoms) and due to this modification the ring has not acidic properties, but on the other hand, it still retains the ability to form hydrogen bonds. The oxytocin analogues containing tetrazole derivatives of aspartic, glutamic and α-aminoadipic acids were obtained by stepwise coupling of Fmoc-amino acid to the growing peptide chain, whereas the analogues in which the Gly-NH_2 residue in position 9 of OT was substituted by tetrazole derivative of glycine (**X** and **XI**) were synthesized by fragment condensation. First, H-Leu-GlyT and H-Leu-GlyT^1CH_3 were synthesized by different methods (see Experimental), and then they were coupled to the [1–7]OT fragment. The purity of all oxytocin analogues prepared was higher than 99% and their structures were confirmed by MS (Table 1).

The analogues were tested for their rat uterotonic *in vitro* and pressor activities and for their affinity to human oxytocin receptor. The data of the bioassays are presented in Table 2.

Replacement of the amide groups of Gln^4, Asn^5 and Gly-$NH_2{}^9$ by tetrazole groups led to analogues **I**–**XI** with low uterotonic activity in comparison to the parent hormone (Table 2). The potency of these analogues increased 2 to 15 times when it was determined in the presence of Mg^{2+} ions. The enhancing effect of Mg^{2+} ions (Munsick, 1960; Krejči & Polaček, 1968) occurs at the receptor level and is not a property of the peptide *per se* (Soloff & Grzonka, 1986). Analogues in which Gln^4 was substituted with tetrazole derivatives of aspartic, glutamic or α-aminoadipic acid (analogues **I**, **V** and **VIII**) had approx. 2–4 % or 4–30% of the uterotonic activity of OT in the absence of Mg^{2+} or in the presence of Mg^{2+} ions, respectively. Analysis of these data revealed that the replacement of amide group by the acidic tetrazolic group is the main reason of the diminished activities of the analogues. The steric effect of the tetrazole group is less important, because the analogues **I**, **V** and **VIII**, which differ in the length of the side chains in position 4, have roughly similar uterotonic activities. The data presented here thus suggest that the interaction of the side chain of Gln^4 with the OTR is influenced mainly by electronic effects and hydrogen bonding capacity of the carboxamide group rather than by steric effects of the side chain.

N-Methylation of the tetrazole ring of the amino-acid residue in position 4 lessened further the uterotonic activities in comparison to the non-alkylated analogues. In the case of N-methylated $Asp(T)^4$ analogues, higher activity was found for the analogue methylated on the N^2 tetrazole atom (compound **III**), whereas with analogues containing an N-methylated Glu(T) residue more active was the analogue methylated on the N^1 atom (compound **VI**). Generally, OT analogues having N-methylated tetrazole derivatives of Asp were more active than the corresponding Glu analogues.

As one could expect, substitution of Aad(T) in position 4 by its D-enantiomer (compound **IX**) led to the analogue which was almost inactive.

The lowest uterotonic activity was found for analogue **IV** that contains the tetrazole derivative of aspartic acid in place of asparagine in position 5. This finding shows that the introduction of the

Table 2. Biological activities of tetrazole analogues of oxytocin

	Analogue	Uterotonic activity (IU/mg)		Pressor activity (IU/mg)	Affinity to human OTR (nM)
		No Mg^{2+}	1 mM Mg^{2+}		
	Oxytocin (OT)	450	450	4.3	2.4
I	$[Asp(T)^4]OT$	17.3±4.5	158±35	0	18.5±2.5
II	$[Asp(T^1CH_3)^4]OT$	4.2±0.4	16.4±7.8	0	26±6
III	$[Asp(T^2CH_3)^4]OT$	11.2±2.3	41.9±15.1	0	25±9
IV	$[Asp(T)^5]OT$	0.08±0.02	1.18±0.40	0	945±85
V	$[Glu(T)^4]OT$	8.9±1.5	47.2±18.5	0	16±0.1
VI	$[Glu(T^1CH_3)^4]OT$	1.8±0.7	8.4±0.8	0	48±7
VII	$[Glu(T^2CH_3)^4]OT$	0.7±0.3	4.3±1.6	0	220±25
VIII	$[Aad(T)^4]OT$	20.1±1.9	80±20	0.19±0.07	17.1±3.7
IX	$[D\text{-}Aad(T)^4]OT$	0.29±0.06	1.8±0.2	0.10±0.02	1496±117
X	$[GlyT^9]OT$	1.7±0.4	1.1±0.3	0	225±80
XI	$[(GlyT^1CH_3)^9]$	5.4±1.1	3.0±1.43	0	405±1

acidic tetrazolic group in this position substantially changes the interaction of the side chain of the appropriate amino-acid residue with oxytocic receptor and confirms the crucial role played by the amide group of Asn[5] in OT structure.

Replacement of glycine amide in position 9 by a Gly(T) residue also gave analogue with very low uterotonic activity. In this case N-methylation did not result in a decrease of the activity. On the other hand, the N[1]-methylated GlyT analogue (compound **XI**) showed uterotonic activity three times higher than the [GlyT[9]]OT (**X**) analogue. The activity of the two latter analogues was not enhanced in the presence of magnesium ions.

There is a good correlation between the uterotonic activity of an analogue measured in the presence of Mg^{2+} ions and its affinity to human oxytocin receptor (Table 2). For analogues **I**, **II**, **III**, **V** and **VIII** the affinities for OTR are approx. 10 times lower than that of the parent hormone. Again, substitution of asparagine in position 5 with the tetrazole analogue of aspartic acid gave analogue **IV** with a very low affinity to OTR.

Only analogues of oxytocin substituted in position 4 by the α-aminoadipic acid residue had residual pressor activity. All other analogues were devoid of pressor activity.

The tetrazole derivatives of Asp, Glu and Gly proved useful in explaining the role of amide groups in the structure–activity relationships of oxytocin. Therefore, they can be useful also in the SAR analysis of other peptides containing asparagine, glutamine or glycine amide.

Acknowledgements

This work was supported by the University of Gdańsk grant DS/8350-5-0131-7, European Social Fund ZPOR/2.22/II/2.6/APR/U/2/05 (stipend to MM) and research project of the IOCB AVCR Z40550506.

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