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Potent and selective oxytocin receptor agonists without disulfide bridges

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ABSTRACT

Oxytocin (OT) is a neuropeptide involved in a wide variety of physiological actions, both peripherally and centrally. Many human studies have revealed the potential of OT to treat autism spectrum disorders and schizophrenia. OT interacts with the OT receptor (OTR) as well as vasopressin 1a and 1b receptors ($V_{1a}R$, $V_{1b}R$) as an agonist, and agonistic activity for $V_{1a}R$ and $V_{1b}R$ may have a negative impact on the therapeutic effects of OTR agonism in the CNS. An OTR-selective agonistic peptide, FE 202767, in which the structural differences from OT are a sulfide bond instead of a disulfide bond, and *N*-alkylglycine replacement for Pro at position 7, was reported. However, the effects of amino acid substitutions in OT have not been comprehensively investigated to compare OTR, $V_{1a}R$, and $V_{1b}R$ activities. This led us to obtain a new OTR-selective analog by comprehensive amino acid substitutions of OT and replacement of the disulfide bond. A systematic amino acid scanning (Ala, Leu, Phe, Ser, Glu, or Arg) of desamino OT (dOT) at positions 2, 3, 4, 5, 7, and 8 revealed the tolerability for the substitution at positions 7 and 8. Further detailed study showed that *trans*-4-hydroxyproline (*trans*-Hyp) at position 7 and γ-methylleucine [Leu(Me)] at position 8 were markedly effective for improving receptor selectivity without decreasing the potency at the OTR. Subsequently, a combination of these amino acid substitutions with the replacement of the disulfide bond of dOT analogs with a sulfide bond (carba analog) or an amide bond (lactam analog) yielded several promising analogs, including carba-1-[*trans*-Hyp^7,Leu(Me)^8]dOT (**14**) with a higher potency (7.2 pM) at OTR than that of OT and marked selectivity (>10,000-fold) over $V_{1a}R$ and $V_{1b}R$. Hence, we investigated comprehensive modification of OT and obtained new OT analogs that exhibited high potency at OTR with marked selectivity. These OTR-selective agonists could be useful to investigate OTR-mediated effects on psychiatric disorders.

Oxytocin (OT), a neuropeptide composed of nine amino acids (AAs) forming a disulfide bridge,[1] is endogenously synthesized in the hypothalamus and secreted from the neurohypophysis to the bloodstream (Fig. 1). OT interacts with the OT receptor (OTR), a typical member of the class I family of G protein-coupled receptors expressed in peripheral tissues (uterus, testis, kidney, heart, and so on) and the central nervous system (CNS).[2] Regarding peripheral actions, OT is clinically used as a labor inducer causing contraction of the uterus. In the CNS, OT is an important hormone for the formation of social attachment in voles.[3] Furthermore, many clinical studies have suggested that OT plays a critical role in social behavior in humans.[4–6] Social information processing in autism subjects is facilitated by intravenous OT administration.[7] Intranasally administrated OT improves emotion recognition in young subjects with autism spectrum disorders,[8] and enhances controlled social cognition in patients with schizophrenia.[9] Low plasma OT levels in autistic children have also been reported.[10] Hence, the therapeutic effects of OT on the brain are expected to contribute to the treatment of autism spectrum disorders and schizophrenia.

As a closely related neuropeptide, arginine vasopressin (AVP) is structurally similar to OT, with differences in only two AA residues at positions 3 and 8. The effects of AVP on emotional expression of fear in rats are opposite to those of OT.[11] However, OT interacts not only with OTR but also AVP 1a and 1b receptors ($V_{1a}R$ and $V_{1b}R$) as a weak agonist in the CNS. Thus, an OTR-selective agonist is necessary to further investigate the effects on the CNS mediated by OTR.

WAY-267464 was previously discovered as a small molecular OTR agonist.[12] WAY-267464 exhibits a functional EC_{50} value of 0.88 μM for OTR, and does not activate $V_{1a}R$ at up to 100 μM, while EC_{50} values of OT for OTR and $V_{1a}R$ are 0.009 μM and 0.060 μM, respectively.[13] However, WAY-267464 does not exhibit the antidepressant-like activity that is seen in OT using a mouse tail suspension test. The $V_{1a}R$ antagonistic activity of WAY-267464 was later indicated.[14]

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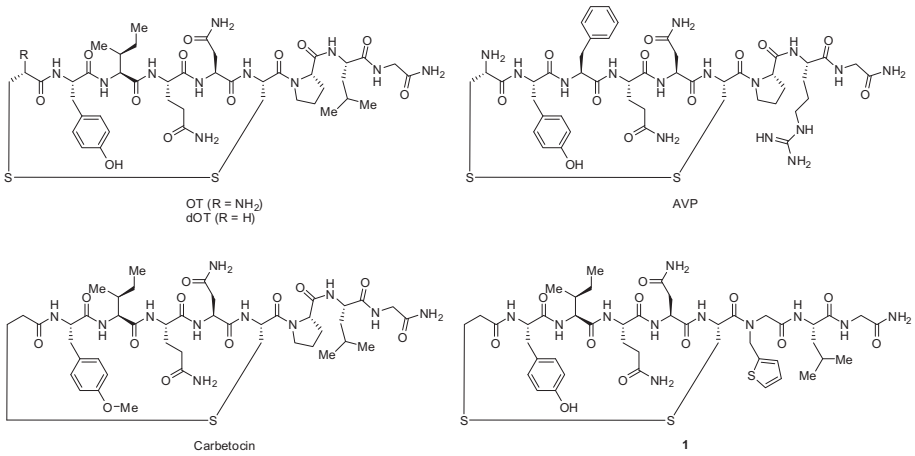


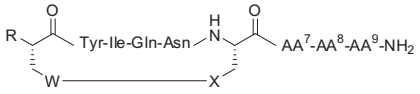
Fig. 1. Structures of OT, dOT, AVP, carbetocin, and analog **1.**

As for peptidic OTR agonists, carbetocin is the only approved OT analog but is a partial agonist, with an 8.5-fold lower potency at OTR than that of OT.[15] Another analog, FE 202767, was found to have EC_{50} values of 0.08 nM, >10,000 nM, and 180 nM for OTR, $V_{1a}R$, and $V_{1b}R$, respectively.[16] The discovery of carbetocin and FE 202767 indicates that potent and selective OTR agonists can be obtained by the modification of OT. Many researchers have studied AA substitutions of OT since the structural identification of OT in 1953. Nevertheless, comprehensive studies to clarify the relationship between AA substitutions and agonistic activities for OTR, $V_{1a}R$, and $V_{1b}R$ have rarely been reported, which motivated us to obtain new OT analogs with potent and selective OTR agonistic activity. Although the activity of OT analogs for the AVP2 receptor (V_2R), which is mainly expressed in peripheral organs such as the kidney,[17] should be considered for clinical application, we have first focused on the validation of OT agonists with selectivity for OTR over V_1R in the brain.

It is known that dOT, an OT analog lacking a free amino group of Cys^1 (Fig. 1), exhibits almost the same OTR activity as that of OT.[18] In this study, dOT was used as a lead peptide to obtain the basic structure-activity relationship of OT. The AA residues at positions 2, 3, 4, 5, 7, 8, and 9 of dOT were replaced with Ala, Leu, Phe, Ser, Glu, or Arg, and the agonistic activities of the obtained analogs were evaluated for OTR, $V_{1a}R$, and $V_{1b}R$ in Ca^{2+} flux assays to clarify what side-chain characteristics (i.e., hydrophobic, aromatic, hydrophilic, acidic, or basic) were preferable for each position, followed by further replacement. In parallel, conversion of the metabolically unstable disulfide bridge in dOT was investigated. On the basis of these results, we designed and synthesized OT analogs by combining AA substitutions and conversion of the disulfide bridge to obtain potent and selective OTR agonists.

The agonistic activities of OT, dOT, and an OTR-selective analog (**1**) were evaluated in Ca^{2+} flux assays as reference compounds.[16,19] As previously reported, OT was a nonselective OTR agonist with EC_{50} values of 8.0 pM, 1500 pM, and 5800 pM for OTR, $V_{1a}R$, and $V_{1b}R$, respectively (Table 1). dOT showed almost the same activity as that of OT, and was used as a lead compound in the study. Compound **1**, with a 2-thienylmethyl-modified Gly residue at position 7, exhibited potent OTR agonist activity (7.3 pM), and was more than 10,000-fold selective versus $V_{1a}R$ and $V_{1b}R$. Thus, we aimed

Table 1
Structures and biological activities of OT analogs with single modifications.



	R	W	X	AA^7	AA^8	AA^9	Agonist activity[a] EC_{50}, pM (95% confidence interval)			Selectivity	
							hOTR	$hV_{1a}R$	$hV_{1b}R$	$hV_{1a}R$/hOTR	$hV_{1b}R$/hOTR
OT	NH_2	S	S	Pro	Leu	Gly	8.0 (6.0–11)	1500 (1200–1800)	5800 (4500–7400)	190	730
dOT	H	S	S	Pro	Leu	Gly	9.1 (4.7–18)	11,000 (9000–14,000)	3200 (1600–6300)	1200	350
1	H	S	S	Xaa	Leu	Gly	7.3(3.6–11)	>100,000	75,000(58,000–98,000)	>10,000	10,000
2	H	S	S	*trans*-Hyp	Leu	Gly	5.3(3.0–9.3)	>100,000	>100,000	>10,000	>10,000
3	H	S	S	*cis*-Hyp	Leu	Gly	7.0 (3.8–13)	>100,000	8200 (5600–12,000)	>10,000	1200
4	H	S	S	Sar	Leu	Gly	6.9 (2.8–17)	>100,000	>100,000	>10,000	>10,000
5	H	S	S	Pro	Leu(Me)	Gly	8.6 (4.6–16)	>100,000	>100,000	>10,000	>10,000
6	H	S	S	Pro	Aoc	Gly	27(16–45)	3100 (900–10,000)	550 (310–990)	120	20
7	H	S	S	Pro	Leu	Sar	120 (65–210)	>100,000	>100,000	>100	>100
8	H	S	S	Pro	Leu	azaGly	7.5 (5.3–11)	70,000 (46,000–110,000)	7300 (4500–12,000)	9300	970
9	H	CH_2	S	Pro	Leu	Gly	6.7 (4.9–9.2)	45,000(37,000–56,000)	6500(4400–9500)	6700	970
10	H	CO	NH	Pro	Leu	Gly	120 (72–190)	>100,000	>100,000	>100	>100
11	H	NH	CO	Pro	Leu	Gly	13,000 (9700–18,000)	>100,000	>100,000	>7.7	>7.7

[a] EC_{50} values of agonist activities were determined as concentrations of peptide analogs that gave half-maximum [Ca^{2+}] mobilizing activities. Xaa, *N*-2-thienylmethylglycine; Sar, *N*-methylglycine, sarcosine; Leu(Me), γ-methylleucine; Aoc, aminooctanoic acid; azaGly, azaglycine.