SELECTIVE OXYTOCIN ANTAGONIST AND ITS USE FOR
ANTAGONIZING OXYTOCIN EFFECTS TESTED BY
BEHAVIORAL STUDIES IN THE OPEN FIELD

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Oxytocin (OXY) is involved in regulation of many central nervous system functions,
however, many of its actions are yet not known. In this study, we used three OT-receptor
antagonists with different structure and selectivity as experimental tool for the study of
spontaneous behavior in the open field. The antagonist with high selectivity (cyclo(1-6)-
β,β-(CH₂)₃Mpa-D-Tyr-Ile-Thr-Asn-Cys-Pro-Orm-NH₂) was prepared in our laboratory. We
found great diversity in the antagonistic effect. Meanwhile grooming was blocked complet-
ely by all antagonists, other spontaneous behavioral parameters were blocked only par-
tially.
Keywords: Behavior; Open field; Grooming; Oxytocin antagonists; Rat.

Oxytocin (OXY) is involved in regulation of many central nervous functions,
which are very extensively studied on animal models, mainly by
behavioral tests which analyze parameters like exploration, memory and
learning, social and maternal behavior etc.¹.

Grooming in rodents represents very sensitive parameter of spontaneous
behavior which is induced among other factors by stress of novelty and
also by OXY. In order to answer a question whether grooming induced by
OXY is caused by stimulation of OXY receptors, we used three different specif-
cic OXY antagonists (see below) and compared their potency to antago-
nize grooming and other parameters of spontaneous behavior, including
exploratory behavior, induced by OXY.

![Chemical Structures]

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Selective Oxytocin Antagonist

Since peptides have very limited penetration through the blood brain barrier, we used as a reference drug non-peptide antagonist L-368,899 (Tocris, UK) (A99). As classical antagonist, we applied clinically used tocolytic atosiban (Polypeptide Labs, Sweden) (ATO) and finally, we used over 100 times more selectively acting OXY antagonist, which was originally prepared by Manning et al.\textsuperscript{2,3} and resynthesized by us (AOA).

**EXPERIMENTAL**

*Synthesis of cyclo[(1-6)β,β-(CH\textsubscript{2})\textsubscript{3}Mpa-v-Tyr-Ile-Thr-Asn-Cys-Pro-Orn-NH\textsubscript{2} (AOA).* The compound was prepared by solid-phase synthesis procedure. \(\beta\)-(4-Methoxybenzyl)mercaptop-\(\beta\)-cyclopentamethylenepropionyl-v-Tyr-(\(t\)-Bu)-Ile-Thr-(\(t\)-Bu)-Asn-Cys(Trt)-Pro-Orn(Boc)-NH\textsubscript{2} was synthesized using the Fmoc-\(t\)-Bu protection protocol on 1.16 g (0.7 mmol) Rink amide resin (Rink resin substitution 0.6 mmol/g). Individual couplings were carried out with the aid of HBTU (2.5 equivalent excess) in 2% HO\textsubscript{B}t in DMF and DIPEA was employed as the base for neutralization and activation. Deprotection step (Fmoc) was done with 20% piperidine in DMF (5 and 30 min). Then washing procedure followed. As the reactor a plastic syringe equipped with polypropylene fritted disc was used. The protected peptidyl resin was further deprotected by HF in the presence of anisole as scavenger. After the reaction was completed, the HF was evaporated in vacuo, the peptide was extracted from the resin with TFA and precipitated with ether. 450 mg of the crude peptide were obtained. Crude reduced \(\beta\)-mercaptop-\(\beta\)-cyclopentamethylenepropionyl-v-Tyr-Ile-Thr-Asn-Cys(SH)-Pro-Orn-NH\textsubscript{2} was oxidized by iodine for 3 h. The solution was pumped through the preparative HPLC column of reverse phase Biosphere C18 (25 \(\times\) 250 mm). The column was washed with 200 ml 1% AcOH and the product was then purified in the gradient of MeOH (A = 1% AcOH in water, B = MeOH, 0–60% in 60 min). 200 mg of the peptide antagonist were obtained. Its purity was evaluated by analytical HPLC and ESI mass spectra.

*Animals.* Male Wistar rats (Velaz, Czech Republic) with starting body weights 200–220 g were used. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals, DHEW Publication, NIH 80-23.

*Treatment.* Saline, oxytocin (OXY), and/or OXY antagonists were injected i.p. in a volume of 2 ml per kg b.w. 1 h before the behavioral testing in the open-field device.

*Behavioral testing.* Spontaneous behavior of rats was video-monitored by an automated activity monitoring system (AnyMaze, Stoelting, USA) in a circular arena with diameter of 150 cm and inner zone diameter of 120 cm. The total number of rears and the total time spent in grooming (the face washing, body and genital licking, body and paw licking and scratching).

*Statistics.* A one-way ANOVA was used. Statistical significance was accepted when \(p \leq 0.05\).

**RESULTS AND DISCUSSION**

Spontaneous behavior in the open field is considered as an indicator of emotionality in laboratory rodents\textsuperscript{4}. The aim of this study was to use OXY antagonists as experimental tool for estimation how these drugs influence the stress of novelty and effects of OXY on behavioral parameters in the
open-field test. Table I summarizes the results of three experiments in which we tested the effect of three different OXY antagonists on spontaneous behavior of rats, on horizontal exploration expressed as total movement distance (TMD) and movement in the central zone (CMD) as an indicator of anxiety; third measured parameter was grooming, which is inherited behavioral effect of OXY and some other centrally acting drugs as alpha-MSH and ACTH.*

In this study, we applied all peptides and non-peptide A99 intraperitoneally; their behavioral effects indicate that all of them penetrate blood brain barrier at least in a small amount.

In all three experiments, we used OXY in the dose of 1.0 mg/kg, which reduced horizontal activity and strongly increased grooming. Movement of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TMD</th>
<th>CMD</th>
<th>Grooming</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAL</td>
<td>23.3 ± 3.1</td>
<td>8.4 ± 2.0</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>OXY</td>
<td>4.5 ± 1.8</td>
<td>0.3 ± 0.1</td>
<td>50.3 ± 2.0</td>
</tr>
<tr>
<td>A99</td>
<td>23.2 ± 3.2</td>
<td>3.7 ± 1.5</td>
<td>5.3 ± 2.0</td>
</tr>
<tr>
<td>OXY + A99</td>
<td>13.8 ± 4.0</td>
<td>1.5 ± 0.5</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAL</td>
<td>25.8 ± 2.9</td>
<td>0.6 ± 0.2</td>
<td>6.5 ± 3.0</td>
</tr>
<tr>
<td>OXY</td>
<td>6.8 ± 1.2</td>
<td>0.2 ± 0.1</td>
<td>16.2 ± 4.7</td>
</tr>
<tr>
<td>ATO</td>
<td>21.8 ± 3.4</td>
<td>2.7 ± 1.1</td>
<td>2.6 ± 1.6</td>
</tr>
<tr>
<td>OXY + ATO</td>
<td>10.1 ± 2.5</td>
<td>0.7 ± 0.2</td>
<td>4.4 ± 3.0</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
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</tr>
<tr>
<td>SAL</td>
<td>20.7 ± 3.0</td>
<td>5.1 ± 1.5</td>
<td>5.4 ± 2.6</td>
</tr>
<tr>
<td>OXY</td>
<td>7.6 ± 1.5</td>
<td>1.2 ± 0.9</td>
<td>32.3 ± 3.0</td>
</tr>
<tr>
<td>AOA</td>
<td>19.4 ± 3.4</td>
<td>0.9 ± 0.9</td>
<td>8.5 ± 5.0</td>
</tr>
<tr>
<td>OXY + AOA</td>
<td>11.5 ± 2.2</td>
<td>2.5 ± 0.7</td>
<td>4.7 ± 3.8</td>
</tr>
</tbody>
</table>

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rats in the inner zone (CMD) was relatively small, nevertheless in all experiments OXY reduced the activity. Due to relatively large SEM it was difficult to interpret data of this parameter. However, the other two parameters were influenced very significantly. Remarkable was the diversity in the antagonistic effect of used drugs on TMD and grooming.

In the first experiment, we used non-peptide antagonist A99. Grooming was antagonized completely while TMD was antagonized by about 50%. In the second experiment, we used ATO which is clinically used non-selective OXY antagonist. Also this drug completely blocked OXY-induced grooming while TMD was blocked by about one third. Finally, in the third experiment, we used highly selective antagonist AOA. Also this drug completely blocked OXY-induced grooming and TMD was blocked just above the level of significance.

Rats grooming belongs to the behavior increased by OXY and also by exposure of rats to a mild stressor, e.g. novelty. Grooming has been proposed to be related to a reduction of arousal during and following stressors. This parameter was antagonized completely regardless the used antagonist. However, none of the used OXY antagonists was able to restore completely TDM and rearing reduction induced by high dose of OXY. This suggests that grooming and other tested behavioral parameters are mediated by OXY-receptors in different localization or conformation and using different signaling pathways for grooming and other behavioral manifestations. Further, due to pronounced behavioral effects it is possible to assume that the used drugs penetrated the blood brain barrier in sufficient amounts after parenteral application.

The results of this study show that in the open-field test OXY-evoked behaviors are indicative of emotionality/anxiety attenuation. The diversity in the intensity of antagonistic action on grooming and other behaviors in the open field deserve further investigation, mainly in the light of possible therapeutic use of various peptides in states where OXY is implicated in the etiology of some psychic disorders.

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