Oxytocin and carbetocin effects on spontaneous behavior of male rats: modulation by oxytocin receptor antagonists

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Abstract

OBJECTIVES: Oxytocin (OXY) in addition to peripheral actions has many central regulatory functions which can be studied on animal models. In the present study we examined in rats, which behavioral actions of OXY and long-acting carba-analog of OXY carbetocin (CBT) in the open-field can be inhibited by OXY-receptor antagonists. Our interest focused on the behavioral patterns considered indicative of anxiety-related behavior. To determine what is the participation of OXY receptor on OXY and CBT induced behavioral changes, we used two peptide and one nonpeptide OXY antagonists differing in selectivity for OXY receptor.
METHODS: OXY, CBT as well as OXY antagonists were injected intraperitoneally, and spontaneous behavior (horizontal and vertical activity, grooming) of Wistar rats was observed in the circular open-field arena 60 min after application of drugs; in some experiments testing was performed without treatment few days after drug administration.
RESULTS: OXY at the dose 0.05 mg/kg increased locomotion indicating anxiety attenuation, but 1.0 mg/kg reduced both locomotion and rearing. CBT in the dose range 0.1–3.0 mg/kg either did not change or increased horizontal activity. The increase in exploration after both peptides persisted for several days. A marked difference in the behavioral effects of the two peptides was grooming enhancement induced by OXY compared with the absence of this effect after CBT. The increase of the activity induced by OXY and CBT indicating anxiolytic-like action was blocked by OXY antagonists. However, the reduction of exploration induced by 1.0 mg dose of OXY was only partially reversed. The OXY induced enhanced grooming was completely antagonized by all used antagonists.
CONCLUSIONS: Behavioral effects of OXY and its antagonists after their i.p. application indicate that they penetrate blood brain barrier. The diversity in potency of OXY antagonists to inhibit grooming and other behaviors induced by OXY suggests that receptors participating in these behaviors may differ in brain localization, receptor conformation and/or in the utilized signaling pathways.

ORIGINAL ARTICLE

INTRODUCTION

Oxytocin (OXY), a nonapeptide synthesized primarily by neurosecretory cells in the mammalian hypothalamus, is well known for its effects on facilitating uterine contractions during parturition and milk let-down during lactation. In the central nervous system OXY acts as a neurotransmitter/neuromodulator and has been shown to regulate a diverse range of central nervous system functions. A growing body of evidence indicates that OXY can modulate neuroendocrine, behavioral and autonomic responses to stress (Petersson *et al.,* 1996; Petersson & Uvnäs-Moberg, 2007; Gimpl & Fahrenholz, 2001). Centrally delivered OXY or its increased level due to steroid manipulation attenuated the stress-induced activity of hypothalamo-pituitary-adrenal axis (Windle *et al.,* 1997; 2006). Findings that OXY attenuates the behavioral reaction of both male and female rats and mice to a stressful event have been interpreted as an anxiolytic-like activity (Windle *et al.,* 1997; 2006; Waldherr & Neumann, 2007; Ring *et al.,* 2006). Furthermore, OXY has been found to be critically involved in mammalian affiliative behaviors, including sexual bonding, mother-infant and adult-adult pair-bond formation (Mc Carthy & Altemus, 1997; Uvnäs-Moberg, 1998).

Based on the evidence from animal studies demonstrating that OXY is implicated in the regulation of species-typical social behavior, the peptide has been proposed as a potential factor in certain psychiatric disorders. OXY neurotransmission may account for several features of autism or obsessive compulsive disorder (Insel *et al.,* 1999; Insel and Young, 2000; Modahl *et al.,* 2002; Hollander *et al.,* 2007; Leckman *et al.,* 1994).

A number of OXY analogs have been designed with the aim to prepare possible substitute agents with changed uterotonic and milk-let down activities. Deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin (carbetocin, CBT) was prepared to be protected from aminopeptidase and disulfidase cleavage (Barth *et al.,* 1974). These changes of the molecule resulted in prolongation of uterotonic activity and the analog is widely used to prevent or treat postpartum hemorrhage (Barth *et al.,* 1974; Hunter *et al.,* 1992; Engstrem *et al.,* 1998; Zingg & Laporte, 2003). In search for new biological agents for treatment of psychiatric disorders in which the role of OXY has been implicated, CBT was suggested as a potential candidate (US Patent, 2005).

In previous studies OXY and CBT attenuated the alteration of spontaneous behavior of rats in the open-field paradigm induced by a restraint stress. However, there were differences in the effects of these peptides: the most apparent one was the absence of grooming enhancement in the CBT-treated rats, an action typical for OXY. Also, higher doses of OXY but not CBT caused pronounced sedation (Klenerova *et al.,* 2009).

In view of the proposed role of OXY in triggering parturition, several antagonists of the hormone have been designed primarily aimed at the uterine receptor. However, OXY antagonists have been shown to inhibit also some of the centrally mediated actions (Ring *et al.,* 2006; Waldherr & Neumann, 2002; Windle *et al.,* 2006; Neumann *et al.,* 2000; Pedersen & Boccia, 2002). In the present study we examined, which of the behavioral actions of OXY and CBT in the open-field can be inhibited by the OXY receptor antagonists. Our interest focused on the behavioral patterns considered indicative of anxiety-related behavior but we were also interested in the sedative effect. We also included recording of grooming as an inherent property of OXY. From the currently available antagonists we have chosen one non-peptide and two peptide compounds differing in the selectivity for OXY versus vasopressin V_{1A} receptors. In spite of the fact that used drugs are peptides, we used them intraperitoneally; this route of application was previously shown to produce behavioral effects (Klenerova *et al.,* 2009).

MATERIAL AND METHODS

Animals

Wistar male rats (VELAZ, Czech Republic) aged around 100 days (250–270 g) were housed by four in individual cages (42 × 26 × 25 cm, Plexiglass) in a room at constant temperature (21±1°C) with a 12L/12D schedule, the onset of the light phase being at 6.00 a.m. The animals were daily handled by the same person and allowed at least 1 week of recovery before behavior testing. Food and water were supplied ad libitum except during actual testing. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NHI 80-23).

Drugs

The following drugs were used: oxytocin (OXY) and carbetocin (CBT) [deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin] from Polypeptide Laboratories, A/S, Czech Republic. As reference drug we used non-peptide antagonist L-368,899 (Tocris, Bioscience, UK) (A99). As classical antagonist we used clinically used tocolytic atosiban (ATO) (Polypeptide Labs AB, Sweden), and finally we used over 100x more selectively acting OXY antagonist cpmProp-D-Tyr-Ile-Thr-Asn-Cys-Pro-Orn-NH_2, originally prepared by Manning et al. (1995) as an acid and resynthesized as an amide (AOA) by M. Flegel.

All drugs were dissolved in saline and injected intraperitoneally in a volume of 2 ml per kg and were administered 60 min before behavioral testing or immediately after termination of stressor exposure.

Stress procedure

Restraint/immobilization stress (IMO) was applied by fixing front and hind legs of the rat with adhesive plaster; then the animal was restrained in a snug-fitting plastic-mesh. This mesh was bent to conform to the size of the individual animal and a bandage fixed this shape

Table 1. Statistical ANOVA values for each behavioral parameter measured in the open-field test for male rats. Statistical differences among individual groups are showed in relevant figures.

	Total movement distance (m)	Total rearing number (n)	Total grooming time (s)
Experiment 1A	F(3,28) = 4.10 $p = 0.015$	F(3,28) = 0.57 $p = 0.64$	F(3,28) = 0.48 $p = 0.69$
Experiment 1B	F(3,28) = 3.27 $p = 0.035$	F(3,28)= 1.10 $p = 0.36$	F(3,28) = 3.44 $p = 0.03$
Experiment 2	F(3,26) = 8.69 $p< 0.001$	F(3,26) = 6.62 $p< 0.001$	F(3,26)= 14.53 $p< 0.001$
Experiment 3	F(3,28) = 7.50 $p< 0.001$	F(3,28) = 7.93 $p< 0.001$	F(3,28) = 3.44 $p = 0.03$
Experiment 4	F(5,42) = 5.68 $p< 0.001$	F(5,42) = 4.87 $p = 0.001$	F(5,42)= 10.24 $p< 0.001$
Experiment 5A	F(4,35) = 2.93 $p< 0.05$	F(4,35) = 0.28 $p = 0.88$	F(4,35)=1.34 $p = 0.28$
Experiment 5B	F(4,35) = 2.77 $p = 0.042$	F(4,35) = 2.00 $p = 0.12$	F(4,35) = 0.80 $p = 0.53$
Experiment 6	F(2,24) = 4.68 $p = 0.02$	F(2,24) = 0.13 $p = 0.88$	F(4,35)= 1.81 $p = 0.18$
Experiment 7	F(2,24) = 4.68 $p = 0.02$	F(2,24) = 0.13 $p = 0.87$	F(2,24) = 0.18 $p = 0.18$

of mesh. During the stress the animals were kept in a vertical position (Klenerova *et al.,* 2007). Stress exposure lasted for 60 minutes. Immediately after stress termination rats received saline or peptides and were returned to the home cage. For the stress application the rats were transferred to a separate room. Two different persons performed the stress procedure and the behavioral testing.

Behavior procedure

Testing was made in the experimental room during the light phase (from 8.00 to 12.00 h a.m.). The room was illuminated by a dim light located on the ceiling. In all experiments rats were randomly assigned to individual groups. Behavior of rats was monitored by an automated activity monitoring system (AnyMaze, Stoelting, U.S.A.) in a circular open-field arena with the diameter of 150 cm; the walls were 50 cm high. The following behaviors were measured: (a) Total movement distance (TMD), expressed in meters (horizontal activity), was recorded automatically; (b) Total number of rearing (vertical activity) and (c) Total time spent in grooming were recorded by the experimenter. At the end of 5 min observation period rats were returned to their home cages. Before using next animal the arena was cleaned with a wet sponge and dried.

Experimental design

Altogether seven experiments were performed. Relevant design of drug treatment as well as of behavioral testing in particular experiments follows.

Experiment 1: Rats were assigned to 4 groups: control – SAL, OXY – 0.05 mg/kg, A99 – 1.0 mg/kg, OXY+A99 – 0.05 mg/kg and 1.0 mg/kg, respectively. Animals were tested 1 h after the treatment (Exp. 1A) and repeatedly 2 days later without additional treatment (Exp. 1B).

Experiment 2: Rats were assigned to 4 groups: SAL, OXY – 1.0 mg/kg, A99 – 1.0 mg/kg, OXY+A99 – both drugs given 1.0 mg/kg. Animals were tested 1 h after treatment.

Experiment 3: Rats were assigned to 4 groups: SAL, OXY – 1.0 mg/kg, ATO – 1.0 mg/kg, OXY+ATO – both drugs 1.0 mg/kg. Animals were tested 1 h after treatment.

Experiment 4: Rats were assigned to 6 groups: SAL, OXY – 1.0 mg/kg, AOA – 0.3 and 1.0 mg/kg, OXY+AOA – OXY 1.0 mg/kg plus 0.3 and 1.0 mg/kg AOA. Animals were tested 1 h after treatment.

Experiment 5: Rats were assigned to 5 groups: SAL, CBT – 0.1, 0.3, 1.0 and 3.0 mg/kg. Animals were tested 1 h after treatment (Exp. 5A) and repeatedly 3 days later without additional treatment (Exp. 5B).

Experiment 6: Rats were assigned to 3 groups: SAL, CBT – 1.0 mg/kg, CBT+AOA – both drugs 1.0 mg/kg dose. Animals were tested on Day 3 after treatment.

Experiment 7: All rats were exposed to IMO and injected immediately after stress termination. Rats were assigned to 3 groups: SAL, CBT – 0.3 mg/kg, CBT+AOA – CBT 0.3 mg/kg plus AOA 0.3 mg/kg. Rats were tested 1 hour following drug treatment.

Statistics

All behavioral data were analyzed with the one-way ANOVA being performed for the treatment effects. In the case of significance a Student-Newman-Keuls test for multiple comparisons followed.

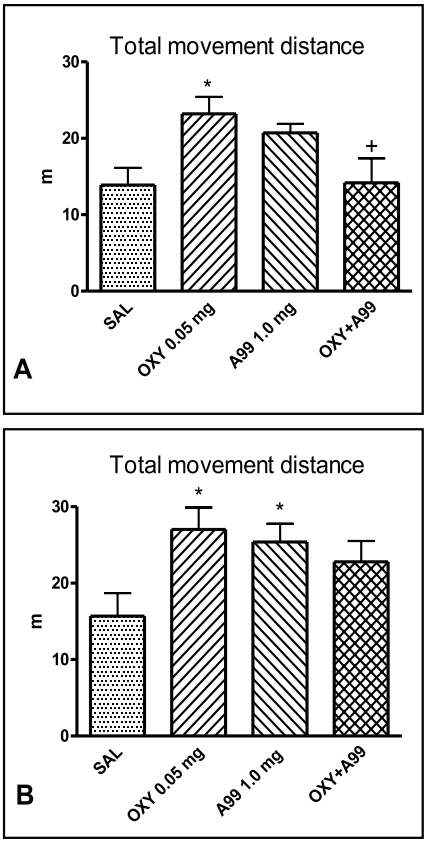

Figure 1. A. Comparison of TMD in the open-field test of rats treated with SAL, OXY (0.05 mg/kg), A99 (1.0 mg/kg) and their combination; testing was performed 60 min after treatment. Significant differences are plotted by * for comparison of appropriate group vs controls (SAL) and + vs OXY. **B.** TMD in the repeated test in the same groups 2 days later without any treatment; * statistically significant vs SAL group ($p<0.05$).

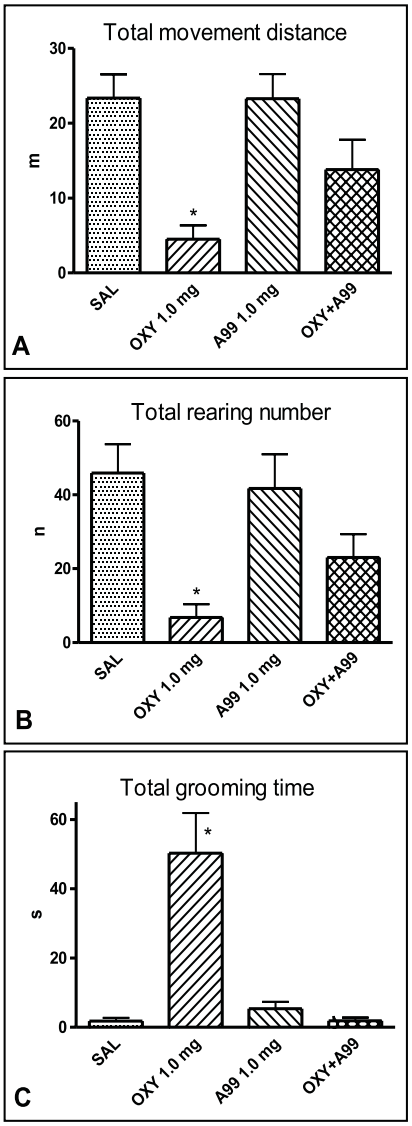

Figure 2. Comparison of three behavioral parameters in the open-field test of rats treated with SAL, OXY (1.0 mg/kg), A99 (1.0 mg/kg) and their combination. **A, B, C:** TMD, rearing and grooming were tested 60 min after treatment. * significant vs SAL and other two groups.

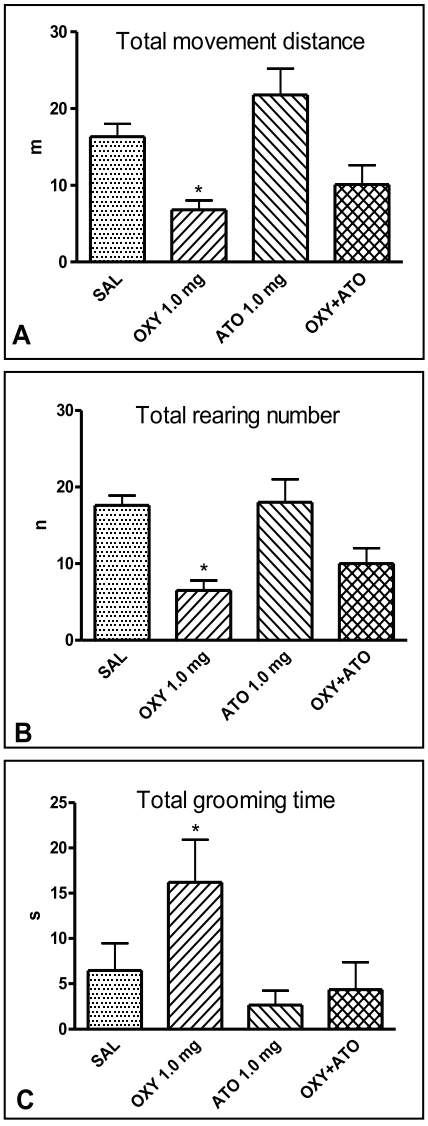

Figure 3. Comparison of three behavioral parameters in open-field test of rats treated with SAL, OXY (1.0 mg/kg), ATO (1.0 mg/kg) and their combination. **A, B, C:** TMD, rearing and grooming were tested 60 min after treatment. * significant vs SAL and other two groups.

RESULTS

Table 1 summarizes statistical ANOVA values of measured behavioral parameters in the individual experiments. Appropriate behavioral values of Experiment 1–7 are presented in Figures 1–7. Values are given as means ± SEM. Differences were considered as significant for $p<0.05$.

Experiment 1: TMD measured in OXY treated animals (0.05 mg/kg) was significantly longer than that in the controls and OXY+A99 treated ones but did not differ from those given A99 alone. No significant differences were found in the rearing number and time spent in grooming (Fig. 1A). Testing of the same animals without additional treatment performed 2 days later revealed persisting increase of TMD in both OXY and A99 treated animals (Fig. 1B). While no significant difference was found in rearing, a growing time spent in grooming was disclosed in animals treated previously with A99 alone.

Experiment 2: TMD in OXY treated animals (1.0 mg/kg) was significantly reduced as compared with other groups (Fig. 2A). Rearing number in OXY treated animals was also decreased: a significant difference was found in comparison with the control and OXY+A99 groups (Fig. 2B). Animals given OXY alone spent significantly more time in grooming than those in the other groups. A99 completely antagonized the effect of OXY on grooming time (Fig. 2C).

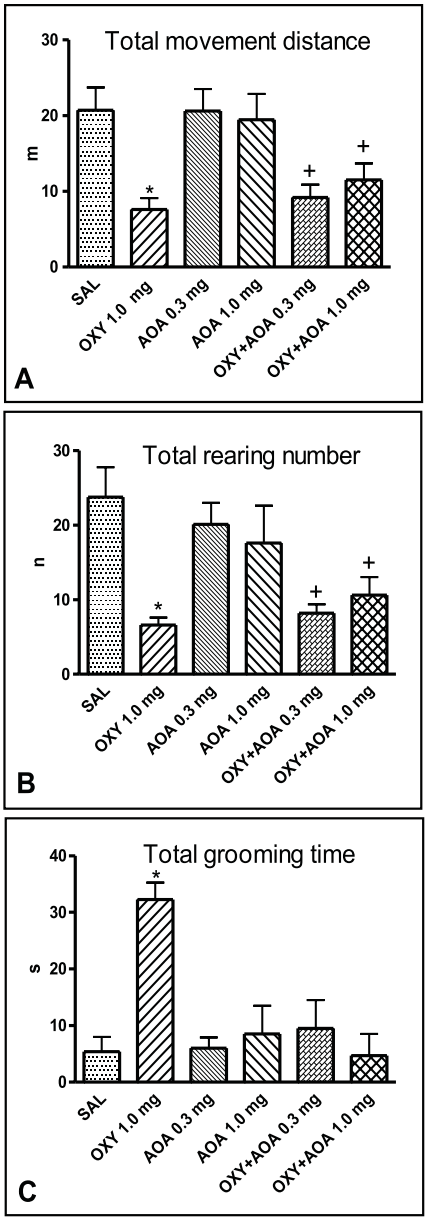

Figure 4. Comparison of three behavioral parameters in the open-field test of rats treated with SAL, OXY (1.0 mg/kg), two doses of AOA (0.3 and 1.0 mg/kg) and their combinations. **A.** TMD 60 min after treatment; * significant vs SAL group; + vs AOA groups. **B.** Rearing number under the same conditions as in A;
* significant vs SAL and AOA 0.3 mg; + vs SAL. **C.** Grooming time under the same conditions as in A;
* significant vs SAL and all other groups.

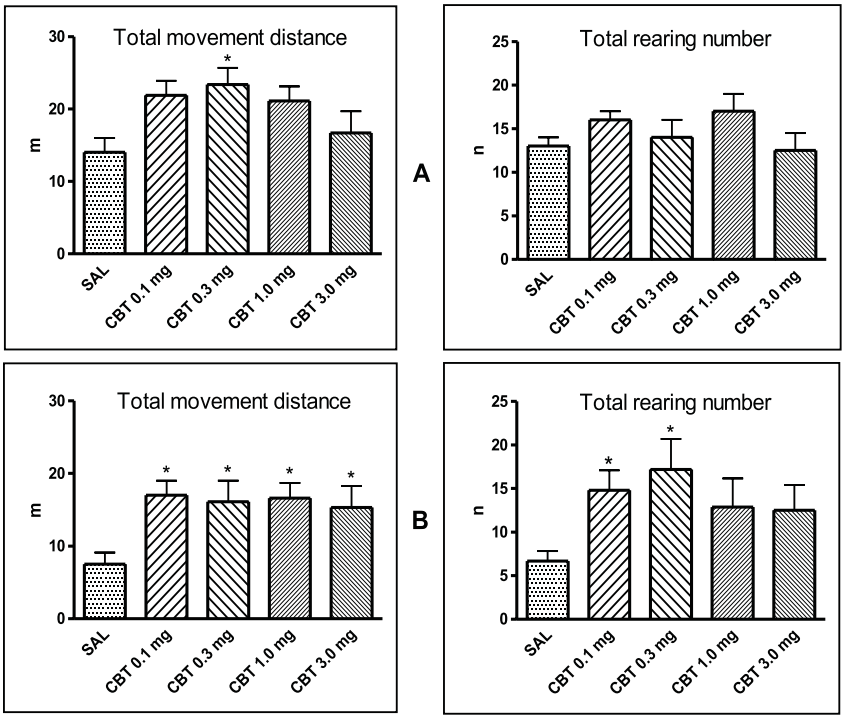

Figure 5. Comparison of TMD and total rearing number in the open-field test of rats treated with SAL and four different doses (0.1-3.0 mg/kg) of CBT 60 min after treatment (**A**) and three days later without any treatment (**B**). * significant vs SAL.

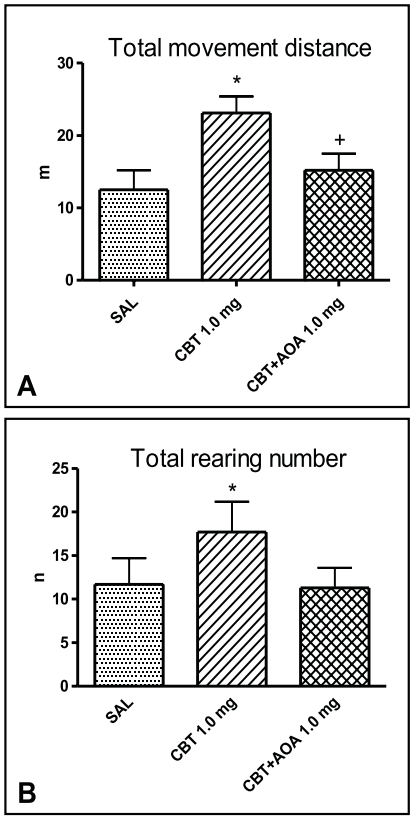

Figure 6. A. The effects of CBT (1.0 mg/kg) and its combination with AOA (1.0 mg/kg) on TMD of rats tested in the open-field three days after drugs application. **B**. Rearing number performed as in A.
* significant vs controls (SAL); + vs. CBT

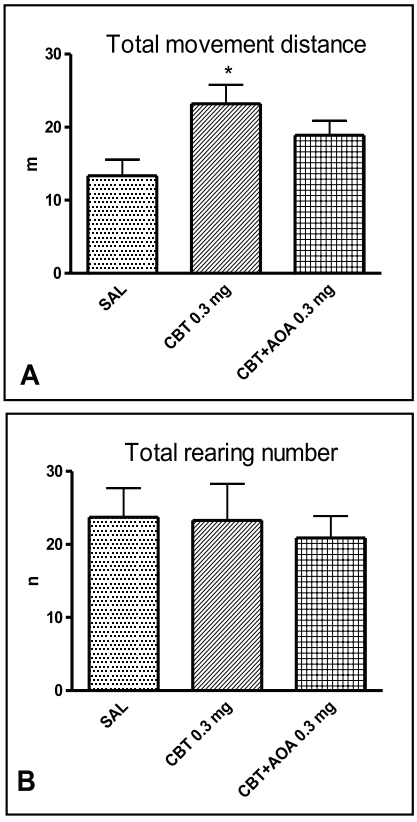

Figure 7. All rats were exposed to IMO for 60 min and immediately after stress termination injected with SAL, CBT and CBT+AOA; behavioral testing followed 60 min later; **A**. TMD after SAL, CBT (0.3 mg/kg) and its combination with AOA (0.3 mg/kg);
* significant vs SAL. **B.** Rearing number in the experiment performed as in A.

Experiment 3: Compared with controls and ATO (1.0 mg/kg) treated animals OXY (1.0 mg/kg) reduced significantly TMD. A significant TMD reduction was also found in animals given OXY+ATO but only in comparison with those given ATO alone (Fig. 3A). The same differences hold true for the rearing number. A significant decrease of rearing number appeared in OXY+ATO group as compared with the controls (Fig. 3B). Animals given OXY alone spent significantly more time in grooming than those in the other groups. ATO completely antagonized the effect of OXY on grooming time (Fig. 3C).

Experiment 4: Compared with the controls, OXY (1.0 mg/kg) reduced significantly TMD as well as rearing number but increased significantly time spent in grooming (Fig. 4 A,B,C). No behavior parameter was changed by AOA doses (0.3 and 1.0 mg/kg). OXY+AOA did not increase the TMD or rearing score of OXY alone, however, it abolished the significant difference compared to the controls (SAL). Prolonged grooming time observed in OXY treated animals was suppressed with parallel AOA administration.

Experiment 5: Compared with the controls, a significantly prolonged TMD was found only in animals given the 0.3 mg/kg CBT dose; the other CBT doses did not reach statistically significant increase (Fig. 5A). No significant differences in the efficacy of different CBT doses were found in the rearing number and time spent in grooming. When tested 3 days later a significant prolongation of TMD was found in all CBT treated groups in comparison with the controls (Fig. 5B). Although there was no overall significant difference in the rearing number, post-hoc t-test revealed a significant increase in animals given CBT at doses of 0.1 and 0.3 mg/kg (Fig. 5B). No difference was found in time spent in grooming.

Experiment 6: Testing on the third day following drug administration showed a significant increase of TMD in CBT (1.0 mg/kg) treated rats compared with controls and CBT+AOA (1.0 mg) group (Fig. 6A). Similar tendency can be observed in the rearing number (Fig. 6B); time spent in grooming was not changed.

Experiment 7: All rats were exposed to restraint stressor. CBT given at the 0.3 mg/kg dose increased significantly TMD compared to controls (Fig. 7A). No differences were found in the rearing number (Fig. 7B) as well as in the grooming time.

DISCUSSION

The aim of the study was to estimate, which of the OXY and CBT actions on the behavior of rats in the open-field situation could be inhibited by the used OXY receptor antagonists. We were primarily interested to find out whether the blockade of OXY receptor interferes with the emotionality and/or anxiety attenuating effects of OXY and presumably also of the analog.

Behavior in the open-field is considered the indicator of emotionality in laboratory rodents, more specifically of fear and anxiety (Prut & Belzung, 2003; Ramos & Mormede, 1998; Ramos *et al.,* 2003; Nosek *et al.,* 2008). An unknown, large, plain and illuminated arena is perceived by the laboratory rodents as an aversive, frightening environment and the behavior of a subject being placed in it reflects the reaction of the subject to that stressful event. It has been widely accepted that this stress of novelty and illumination induces anxiety-like behavior expressed as inhibition of locomotion and rearing; the increase resulting from the effects of clinically effective anxiolytic drugs, such as benzodiazepine receptor full agonists, then is considered to reflect lowering of the anxiety level (Carli *et al.,* 1989; Ramos & Mormede, 1998; Prut & Belzung, 2003). Anxiolytic-like effects of OXY were obtained in the open-field and in some other used animal models of anxiety: both central and systemic injections of OXY have been shown to attenuate anxiety-like behavior in elevated plus- or zero-maze, in black-white box or in paradigms employing punished crossings like four-plate test (Waldherr & Neumann, 2007; Neumann, 2002; Windle *et al.,* 1997; Ring *et al.,* 2006; Uvnäs-Moberg *et al.,* 1994). The attenuation of anxiety could be blocked by several OXY antagonists mostly centrally administered (Ring *et al.,* 2006; Waldherr & Neumann, 2007; Windle *et al.,* 2006; Neumann *et al.,* 2000).

In the present experiments OXY in 0.05 mg/kg dose increased the locomotor activity, indicating thus a disinhibitory, anxiolytic-like action and this effect persisted in the second trial performed 48 hours later (Fig. 1A, 1B). This finding is in agreement with the reported OXY anti-stress effects sustained for several days after repeated OXY treatment (Uvnäs-Moberg, 1998). In this study the antagonist A99 given simultaneously with OXY reversed the increase of TDM, but only on the first day; the increased TDM in the A99 group on the second trial is difficult to explain (Fig.1A, 1B). In contrast to the enhancement of locomotion after the low OXY dose, the administration of 1.0 mg/kg caused a strong depression of both ambulation and rearing in all experiments. A similar reduction in locomotion has been reported in situation with OXY administered either subcutaneously (0.25 and 1 mg/kg) or intracerebrally (1 μg/kg) in rats and this effect was interpreted in terms of sedative effect. This effect could not be reversed by the used antagonist (Uvnäs-Moberg *et al.,* 1994; Uvnäs-Moberg, 1997; 1998). In our experiments decline of TDM and rearing was inhibited by all three used antagonists, but in neither case the behaviors returned to control values. In contrast, the large increase of grooming activity elicited by OXY was completely blocked. The concomitance of locomotor depression with the enhancement of grooming behavior indicates that most probably the locomotor slowdown due to the higher OXY dose was not caused by an impaired motor coordination.

CBT administered in the dose-range 0.1-3.0 mg moderately increased TDM without causing reduction even after the highest dose. The enhancement of horizontal and vertical activities was not only present but even more pronounced in repeated testing after 48 hours (Fig. 5). Similarly, the increase in both exploratory parameters after 1.0 mg/kg CBT was observed on the third day after administration (Fig. 6). No dose of CBT significantly increased grooming. The absence of this behavior in the CBT effect is surprising, as grooming activity is an inherent property of OXY molecule. In our previous study CBT in the doses 0.3 and 1 mg/kg, prevented restraint stress induced locomotion reduction (Klenerova *et al.,* 2009). As expected, in the current investigation CBT increased the ambulation of rats exposed to the stressor. This increase was inhibited by the antagonist AOA.

The sustained effect of OXY has been observed in several experimental paradigms. For example, five days treatment period with OXY gives rise to sedation, lowering of blood pressure or increased withdrawal latency in tail flick test and these effects persist for several weeks. Also, they could not be reversed by OXY antagonists (Uvnäs-Moberg, 1997; Agran *et al.,* 1995). On the other hand, central OXY stimulates under certain experimental conditions rat female sexual behavior and this effect lasting for several hours can be blocked by an OXY antagonist (Pedersen & Boccia, 2002). The mechanisms underlying the persisting effects of OXY, a peptide with a half-life of minutes, presents an important feature of the hormone actions and certainly deserves further investigation.

The increase or decrease of exploration by OXY depending on the dose raises a question if the two opposite effects are mediated by the same OXY receptors. The doubt is further strengthened by the found only partial reversal of sedation, especially in light of the complete blockade of grooming. In contrast to OXY, CBT in the used doses did not reduce locomotion and also did not significantly influence grooming. A possible reason may be differential dose-response curve of the two peptides, caused among others by the longer half-life of the analog (Barth *et al.,* 1974; Hunter *et al.,* 1992). Also, CBT displays effects on the isolated rat uterus and myometrial strip indicating the properties of a partial agonist/antagonist (Barth *et al.,* 1974; Engstrem *et al.,* 1998). OXY receptor belongs to the G-protein coupled receptors, and unlike the receptors for the structurally related peptide vasopressin, OXY actions are mediated by only one type of receptor (Zingg & Laporte, 2003). In displacement studies using recombinant oxytocin/vasopressin V_2 receptor construct, the binding profiles for OXY and CBT were found to be similar (Gimpl *et al.,* 2005).

Rat grooming belongs to the behavior elicited by several types of stressors and also by intracerebral injections of OXY, α-MSH and ACTH; OXY activates and prolongs grooming not only in rats exposed to a mild stressor, e.g. novelty, but also in resting animals. Grooming has been proposed to be related to a reduction of arousal during and following stressors (Gispen & Isaacson, 1981; Van Erp *et al.,* 1993; 1994; Drago *et al.,* 1986). If we accept that the found increase in exploration evoked by the analog both in the non-stressed and restraint stress exposed rats can be interpreted in terms of anxiolytic-like action; then, the absence of grooming enhancement suggests that this behavioral pattern did not play an important role in the anxiety attenuation under the used experimental conditions. To assess the exploratory behavior more in detail, we included also the rearing activity. While this behavioral variable was regularly suppressed by the sedative effect of 1.0 mg/kg of OXY, the increase in TMD by low dose of OXY or by CBT was only inconsistently copied by the increase of vertical movements. One of the reasons for the discrepancy may be that the rearing on hind-limbs in a novel situation has been shown to represent a non-selective attention that is rather sensitive to stimuli of differing properties and salience, which are not easy to maintain under the control (Fresiello *et al.,* 2002).

In the present study all drugs were administered intraperitoneally, raising thus question if their action occurs in the central nervous system. It has been reported, that about 0.1% of systemically delivered OXY passes the blood-brain barrier in guinea pigs (Jones & Robinson, 1982). Restraint stress induced a higher accumulation of intracarotidally injected CBT in the rat brain in comparison with the controls (Dvorska *et al.,* 1992). The used antagonists were designed to compete on uterine OXY receptors and may display differential OXY receptor selectivity (e.g. Manning *et al.,* 1995; Pedersen & Boccia, 2002). Nevertheless, they were capable to completely block the OXY-enhancement of grooming, which is considered to represent an action of the hormone in the brain. Accordingly, we assume that the used drugs penetrated the blood-brain-barrier. The non-penetrant OXY antagonist blocked the anxiolytic effect of OXY only if centrally administered (Ring et al. 2006).

In conclusion, this study shows that in the open-field test OXY and CBT evoked behaviors indicative of emotionality/anxiety attenuation. Although there were some differences in the effects of the peptides, their anxiolytic-like and/or stress attenuating action could be inhibited by three different OXY receptor antagonists. The findings support the notion that CBT may influence the specific sites of OXY in the brain, where the hormone is supposed to exert a regulatory role on fear or stress elicited reactions. Furthermore, these effects of CBT can be viewed as supporting the notion that the drug may be considered a candidate for therapy of psychiatric disorders, where OXY is implicated in their etiology (US Patent, 2005).

Acknowledgements

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