INTRODUCTION

Oxytocin (OXY) is a nonapeptide synthesized primarily by neurosecretory cells in the mammalian hypothalamus. It is well known for its role during parturition and lactation. In the central nervous system OXY acts as a neurotransmitter/neuromodulator and has been shown to modulate a variety of neurophysiological phenomena (1). Among neuropeptides and neurotransmitters in the paraventricular nucleus, corticotropin-releasing hormone, vasopressin and OXY appear to play prominent roles in the responses to various stressors (2-6). OXY decreased blood pressure, reduced corticosterone level and increased insulin and cholecystokinin levels in both male and female rats (7, 8). Centrally delivered OXY or its increased levels due to steroid manipulation attenuated the hypothalamo-pituitary-adrenal axis activity induced by both psychological and physical stressors like white noise, exposure to an elevated platform or forced swimming (4, 5, 9, 10). 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 action (9-13). Further, in animal and human studies OXY has been found to be involved in affiliative behaviors, including sexual or maternal behavior and some other aspects of social attachment (14-16).

A number of OXY analogs have been designed with the aim to prepare possible substitutes with changed uterotonic activities. Deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin (carbetocin, CBT) was prepared to be protected from aminopeptidase and disulfidase cleavage (17, 18). These changes of the OXY molecule resulted in prolongation of uterotonic activity of CBT (17, 19, 20).

In our previous studies restraint/immobilization stress (IS) one hour in duration exerted both short- and long-term alterations of spontaneous behavior in Wistar, Sprague-Dawley or Lewis rats in the open-field test (21-23). In view of the reported OXY involvement in stress coping we hypothesized that OXY and CBT may influence behavioral responses to this type of the stressor.

The aim of the present study was to investigate the impact of post-stress OXY and CBT intraperitoneal administration on behavioral changes in rat behavior in the open-field. Wistar male rats were exposed to restraint for 1 hour; saline or drugs were administered intraperitoneally immediately after stress termination. Recording of the exploratory activity in the open-field started 60 min later. To explore the possibility of persisting effects of stress and/or drugs, the procedure was repeated for three consecutive days. Restraint moderately suppressed locomotion and rearing, and increased grooming. OXY in 0.3 mg/kg dose showed a tendency to restore the suppressed exploratory activity. In contrast, 1 mg/kg dose potentiated the stress-induced behavioral deficit. Both OXY doses slightly increased grooming. CBT in the same two doses restored the stress-induced deficits in locomotion and rearing but did not influence grooming. The locomotor depression after 1 mg dose of OXY was found also in non-stressed rats in contrast to the increased activity after CBT. The data support the view that post-stress administered CBT exerts a significant effect on the stress-altered spontaneous behavior.

Keywords: carbetocin, oxytocin, restraint stress, locomotion, rearing, grooming
MATERIALS AND METHODS

Animals

Male Wistar rats (Velaz, Czech Republic) with starting body weights of 250-265 g were used. They had free access to the standard pellet food (ST1, Velaz, Czech Republic) and water. Rats were housed in a room maintained on a 12-h light/dark cycle (lights on 6-18) and temperature 22 + 1 °C. Four rats were housed per cage (42 cm x 26 cm). Several days before the start of experiment rats were daily handled. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NIH 80-23).

Drugs

The following drugs were used: oxytocin and carbetocin [deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin] from Polypeptide Laboratories, A/S, Czech Republic. Both peptides were dissolved in saline and each dose was injected intraperitoneally (i.p.) in 2 ml/kg b.w.

Stress procedure

Restraint/immobilization stress (IS) 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 of mesh. During the stress the animals were kept in a vertical position (23). 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. The whole procedure was repeated in the same way for three consecutive days.

Behavioral measurements

Behavior of rats in the open-field was video-monitored by an automated activity monitoring system (AnyMaze, Stoelting, U.S.A.) in a circular arena with the diameter of 150 cm; the walls were 50 cm high. Total movement distance was recorded automatically; an experimenter measured the total number of rearings (rearings) and the total time spent in grooming (the face washing, body and genital grooming, body and paw licking and scratching).

Behavioral testing started 1 hour after peptides or saline administration either in the stressed or unstressed rats. At the end of 5-min observation period rats were returned to their home cages and the arena was cleaned with a wet sponge and dried. In all experiments rats were randomly assigned to control and experimental groups (N = 8). The experiments were performed from 8 a.m. to 1 p.m. in a separate room illuminated by a fluorescent light located on the ceiling.

Experimental design

Experiment 1: Rats were assigned to four groups: CO – controls, injected with saline; IS – rats were exposed to IS; IS+OXY – rats were exposed to IS and injected with 0.3 mg/kg of OXY; IS+CBT – rats exposed to IS and injected with 0.3 mg/kg of CBT. The procedure was repeated for three consecutive days.

Experiment 2: The same procedure as in Experiment 1, also repeated for three days; the dose of both peptides was 1.0 mg/kg.

Experiment 3: In unstressed rats saline or peptides in 1.0 mg/kg were injected 1 hour before behavioral testing.

Statistical analysis

Behavioral data were analyzed with a two-way ANOVA being performed for day and treatment effects. A one-way ANOVA was done to compare differences among groups within a given trial (Exp. 3). In the case of significance a Newman-Keuls post-test followed. Always, differences were considered as significant for p<0.05. Data are presented as means ± S.E.M.

RESULTS

Experiment 1: effects of OXY and CBT in the dose 0.3 mg on stressed rats

The results are summarized in Fig. 1. The overall analysis on the total distance traveled revealed a significant effect of treatment [F(3,84) = 43.9, p<0.0001] and a significant effect of day [F(2,84) = 7.2, p<0.001], but no significant effect of treatment x day interaction [F(6,84) = 1.8, p = 0.1]. In comparison with the controls IS treated rats exhibited conspicuous reduction of the locomotion on Day 1, 2 and 3. The same holds true for rats subjected to IS+OXY on Day 2 and 3. No significant difference was found between the controls and IS+CBT treated rats. A comparison of IS and IS+OXY treated rats showed a significantly decreased locomotion of IS rats only on Day 1. Finally, compared with both IS and IS+OXY treated rats, those subjected to IS+CBT treatment exhibited significantly higher locomotion on Day 1, 2 and 3. The overall analysis on the rearing number revealed a significant effect of treatment [F(3,84) = 0.2, p = 0.9999], but no significant effect of day [F(2,84) = 0.31, p = 0.74] and no significant effect of treatment x day interaction [F(6,84) = 0.94, p = 0.47]. Compared with the controls, a significantly reduced rearing was found only in IS treated rats. In comparison with IS+CBT treated rats a significantly reduced rearing was in IS group (p<0.001) and in IS+OXY group on Day 1. The overall analysis on the grooming time revealed a significant effect of treatment [F(3,84) = 26.0, p<0.0001], a significant effect of day [F(2,84) = 3.3, p = 0.04] and a significant interaction effect [F(6,84) = 5.8, p<0.0001]. Evidently, IS treated rats exhibited more grooming than the controls on Day 1, 2 and 3. The same holds true for IS+OXY treated rats on Day 2 and 3. No difference in the grooming was between the controls and IS+CBT treated rats. On Day 3, the IS+OXY group exhibited extremely high grooming when compared with both IS and IS+CBT group.

Experiment 2: effects of OXY and CBT in the dose 1.0 mg on stressed rats

The results are summarized in Fig. 2. The overall analysis on the distance traveled revealed a significant effect of treatment [F(3,84) = 14.8, p<0.0001], but no significant effect of day [F(2,84) = 1.2, p = 0.3] and of treatment x day interaction [F(6,84) = 0.87, p = 0.5]. In comparison with controls a significant reduction of the locomotion was found in rats subjected to IS on Day 3, in IS+OXY treated rats on Day 1, 2 and 3, and in IS+CBT treated rats on Day 1. No significant difference in the locomotion was between IS and IS+OXY as well as between IS and IS+CBT treated rats. IS+CBT treated rats exhibited significantly higher locomotion on Day 3 when compared with IS+OXY treated ones. The overall analysis on the rearing number revealed a significant effect of treatment
F(3,84) = 10.6, p < 0.0001, but not of day \[ F(2,84) = 0.22, p = 0.8 \] and of treatment x day interaction \[ F(6,84) = 0.96, p = 0.4 \]. There was no significant difference between the controls and IS treated rats, and between the controls and IS+CBT treated ones. IS+OXY treated rats exhibited significantly lowered rearing when compared with the controls on Day 1, 2 and 3. No significant difference in the rearing was found between IS and IS+OXY and between IS and IS+CBT treated rats. A significantly higher rearing was in IS+CBT treated rats when compared with the controls on Day 1, 2 and 3. No significant difference in the rearing was found between IS and IS+OXY and between IS and IS+CBT treated rats. A significantly higher rearing was in IS+CBT treated rats when compared with the controls on Day 3. The overall analysis on the grooming time revealed a significant effect of treatment \[ F(3,840) = 9.8, p<0.0001 \], but not of day \[ F(6,84) = 0.03, p = 0.97 \] and of treatment x day interaction \[ F(6,84) = 1.6, p = 0.15 \]. In comparison with the controls, a significantly increased grooming was in IS treated rats on Day 2 and in IS+OXY treated rats on Day 1 and 2. No difference was found between the controls and IS+CBT treated rats. IS+OXY treated rats exhibited significantly increased grooming on Day 1 when compared with both IS and IS+CBT treated rats.

Experiment 3: effects of OXY and CBT in the dose 1 mg on not stressed rats

The results are summarized in Fig. 3. The one-way ANOVA revealed a significant effect in all three measured behavioral parameters: for locomotion \[ F(2,20) = 19.0, (p<0.001) \], for rearing \[ F(2,20) = 4.3, (p=0.028) \], for grooming \[ F(2,20) = 4.7, (p<0.02) \]. OXY in 1 mg/kg dose reduced locomotion (p<0.01) and rearing (p<0.05) but significantly increased grooming (p<0.05) compared with both control and CBT treated rats. The
only significant difference between CBT and controls was found in higher locomotion in CBT rats ($p<0.01$). In a separate experiment CBT in 3 mg/kg dose did not change any of the observed behavioral parameters (data not shown).

**DISCUSSION**

The main finding of this study is the modulatory effect of OXY and CBT on alteration of rat spontaneous behavior in the open-field induced by a restraint stressor. In agreement with our previous results (22, 23) IS mildly reduced locomotion and rearing; repeated IS produced neither progressive suppression of exploratory activity nor indications of habituation. The action of OXY on the suppressed locomotor and rearing activity varied depending on the dose used: while the effect of the 0.3 mg dose indicated a tendency to reverse the stress-induced suppression, the 1 mg dose further decreased exploratory behaviors of IS treated animals. CBT in both doses restored the reduced locomotion and rearing of IS treated rats to control levels.

The deficit in exploratory behavior produced by stressors like restraint or inescapable foot-shock was observed in several studies (24-27). It has been repeatedly argued that the stress-induced behavioral sequelae seen in behavioral models like open-field, elevated plus maze or black-white box, could reflect altered psychological functions such as increased arousal, altered emotionality associated with fear and/or anxiety and changes in motivation (27-30). Behavioral studies employing similar approach indicate that OXY exerts anxiolytic-like action (9-11, 13). In view of these findings it is feasible to speculate that the attenuation of IS stressor-induced behavioral suppression, seen in the present experiments after the lower dose of OXY, may reflect a modulatory effect of the peptide on altered emotionality and anxiety. However, the 1 mg dose of OXY further reduced the stress-suppressed exploratory behaviors. Similar reduction in locomotion and rearing was observed with OXY subcutaneously administered in 0.25 and 1 mg/kg doses in unstressed rats and was interpreted in terms of sedative effect (11). In agreement with those results, attenuation of exploratory activity appeared also in the present study, when the 1 mg dose was given to unstressed rats.

Calmness and sedation as a result of central release of OXY have been suggested in connection with maternal and sexual behavior (1, 13). CBT in both doses reversed suppressive effect of the used stressor. While CBT in 1 mg/kg dose increased locomotion, the higher dose (3 mg) had no enhancing effects on the behavioral parameters. Surprisingly, neither of the used CBT doses increased grooming. Rat grooming belongs to the behaviors elicited by mild stress and also by intracerebral injection of 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 (see 1, 31-34). In the current study grooming activity increased after IS alone, although the enhancement failed to reach significance invariably, due to the large variance in the data. For similar reasons also grooming enhancement elicited by both doses of OXY, in comparison with control and IS groups, did not reach significance on all days.

In the non-stressed animals the 1 mg/kg b.w. dose of OXY produced significant enhancement of grooming simultaneously with the locomotor suppression. The specificity of central OXY effects was tested by the use of non-peptide OXY antagonist L 368899. In favor for the specificity of OXY effect (1 mg/kg b.w., i.p.) is the finding that L 368899 (1 mg/kg b.w., i.p.) attenuated the suppression of locomotor activity and completely abolished the strong grooming augmentation (unpublished results). In contrast to OXY, CBT did not change at all the grooming score in comparison with control or IS groups and the absence of effect on grooming appeared also in unstressed rats. This may indicate a differential binding of the two molecules to central nervous system OXY receptors mediating some of the effects of stress on the behavior. However, the findings that the attenuating action of OXY on some of the stress-induced physiological and behavioral sequelae cannot be blocked by some OXY antagonists suggest an existence of possible unidentified receptor subtype in the brain (16, 35).

**Fig. 3.** The effect of OXY and CBT (in both cases 1.0 mg/kg b.w.) on total distance travelled, rearing number and grooming time in unstressed rats. Behavioral testing was performed 60 min after peptides administration. Statistical significance ($p < 0.05$): * vs Control group (CO), + vs OXY treated group.
OXY receptors in several regions of the limbic system appear to be involved in functions such as social recognition, sexual and maternal behavior or feeding (1, 13, 16, 36). Based on evidence from animal studies demonstrating the effects of OXY on the normal expression of the mentioned social behaviors, it has been suggested that altered OXY levels may be related to several features associated with autism and also obsessive compulsive disorders (37-39). In search for new biological agents for treatment of psychiatric disorders in which abnormal OXY levels are implicated, CBT was suggested as a potential candidate (40). However, the present results indicate possible differences between the actions of the hormone and the analog. Some results showed differential mode of action of OXY and CBT also in the periphery. For example, in comparison with OXY, the analog displays a protracted action on the rat uterus in vivo, but possesses decreased activity on the isolated rat myometrial strip indicating partial agonist/antagonist type of action (17, 19, 20).

It has been reported that about 0.1% of systemically administered OXY passes the blood-brain barrier in guinea pigs (41). Restraint stress induced a higher accumulation of intracarotidally injected CBT in the rat brain in comparison with the controls (42). Therefore, we assume that the effect of the intraperitoneally given peptides in this study was mediated via the central nervous system.

In summary: CBT and partly OXY prevented the locomotion and rearing deficit induced in rats by a restraint stress; both peptides differentially affected grooming. These effects support the notion that CBT acts on the stress-induced behavioral disorders, where intracerebrally released OXY is supposed to exert a regulatory role. Further investigations are needed to determine the specificity, potency and duration of central action of OXY and CBT. The data support the view that post-stress administered CBT exerts a significant antistress effect on the stress-altered spontaneous behavior. It could be hypothesized that also long-term consequences of stress, e.g. posttraumatic stress disorder, may be influenced by OXY derivatives.

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