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Effects of Oxytocin and Thyroliberin on Anxiety in Male White Mice in Social Stress

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The effects of intranasal administration of oxytocin and thyroliberin solutions on changes in behavior in rats after moderate social stress were studied. Investigations were carried out on male Wistar rats (n = 100). Animals of experimental group 1 received intranasal oxytocin at a dose of 0.25 IU in 20 μl bilaterally and those of experimental group 2 received thyroliberin at a dose of 10^{10} mmol in 10 μl, while control animals received the same volume of physiological saline. Animals were exposed to moderate social stress for 1 h starting 15 min after substance administration. Animals were tested in an elevated plus maze (EPM) after a further 3 h. These experiments showed that administration of oxytocin led to a decrease in the level of anxiety in social stress as compared with controls. Administration of thyroliberin had no effect on stress-induced changes in behavioral parameters.

Keywords: oxytocin, thyroliberin, anxiety, social stress.

There are no doubts as to the relevance of the challenge of stress and the search for stress-protective agents [Batuev et al., 2000; Grigor'yan and Gulyaeva, 2015; Kalinina et al., 2012; Kudryashova and Gulyaeva, 2016]. There is an ever-continuing need for studies of the mechanisms regulating behavior, including at the hormonal level, in social interactions. We have previously demonstrated that the neuropeptide thyroliberin (thyrotropin-releasing hormone, TRH) given intranasally to female white rats at ultralow doses has an anxiolytic effect after stress induced by painful electric shock. We found that the specific action of TRH was limited to altering the level of anxiety, as it did not extend to the whole spectrum of behavioral reactions. In particular, TRH had no influence on stress-induced decreases in movement and exploratory activity [Vinogradova et al., 2014]. The clear specificity of the stress-protective effect of TRH raises the question of the relationship between the anxiolytic properties of this peptide and the nature of the stressor, i.e., its influence on behavior on exposure to other stressors, particularly social stress. Among the hormones associated with regulating social interactions is oxytocin (OT) [Kosfeld et al., 2005; Kumsta and Heinrichs, 2013; Neumann, 2008]. The aim of the present work was to study the influences of intranasal administration of TRH and OT on anxiety in rats after social stress induced by transient alteration to the established social composition of a group of animals.

Methods. All experiments were performed in compliance with international standards for biomedical research using animals: the European Convention for the Protection of Vertebrate Animals Used for Experimentation and Other Scientific Purposes, 1986, and the "Regulations for Laboratory Practice in the Russian Federation, approved by Order of the Ministry of Health of the Russian Federation, No. 708n, of August 23, 2010.

Experiments were performed on male Wistar rats (n = 100) with mean weight 170 g and aged two months at the beginning of the experiments. Animals were kept in standard conditions with free access to feed and (dry combined feed for rodents) and water in groups of five individuals per cage. Before experiments started, animals were

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exposed to a handling procedure [Vinogradova and Chaadaeva, 1994]. The criterion for acclimation to the experimenter was food consumption by animals in the experimenter's hands.

Animals were then tested in the elevated plus maze (EPM, Open Science, Russia). This test is currently regarded as one of the most suitable for assessing anxiety [Pellow et al., 1985]. Anxiety as defined by this method reflects the natural fear of heights and open spaces in rodents. The time spent in the open arms of the maze reflects the level of the animal's anxiety: increases in the time spent in the open arms provide evidence of decreased anxiety levels [Pellow et al., 1985]. This test is now widely used in studies addressing anxiety and the testing of anxiolytic drugs: an analysis of published scientific studies for the period 1990–2011 indicated that the EPM test was used in around 50% of investigations [Haller et al., 2013].

Animals were placed in the center of the EPM in a position in which the head was oriented towards the open arm. The time spent in the open arms of the EPM was recorded, along with the number of maze sectors crossed during the test, the number of vertical rearings, and the number of hangings from the open arms of maze, as well as the duration of grooming reactions and the number of boluses. Testing of each animal took 5 min. After testing of each animal, the maze was cleaned with hydrogen peroxide solution to remove odors. All animals were tested between 13:00 and 17:00.

The data obtained during the first test were used to divide the animals into four groups – experimental group 1 ($n = 25$), experimental group 2 ($n = 25$), the control group ($n = 25$), and a group of intact animals ($n = 25$). There were no significant between-group differences in mean measures of anxiety and movement activity.

A month after initial testing in the EPM, the effects of TRH and OT on social stress-induced increases in anxiety were investigated. Animals of experimental group 1 received OT (Gedeon Richter) at a dose of 0.25 IU in a volume of 20 μl bilaterally, the dose given being selected on the basis of analysis of published data [Kovalenko et al., 1997; Kent, 2015]. Animals of experimental group 2 received intranasal TRH (Hybio Pharmaceuticals Co. Ltd.) in 10 μl containing 10^{-10} mol. This concentration is known not to induce the release of hypophyseal hormones [Chepurnov et al., 2002]. The control group received the same volume of intranasal physiological saline. Intact animals received no treatments.

Animals were subjected to social stress 15 min after substance administration. The method used for creating social stress consisted of combining animals of two standard home cages (40 × 30 × 15 cm) into one, larger, cage (56 × 35 × 20 cm). The animals were placed back in their home cages after 1 h. All animals were tested in the EPM 3 h later. All animals were tested in the elevated round maze (NPK Open Science, Russia) at one day to identify any possible continuing effect of the agents given. The construction of the round maze, the test method, and the interpretation of the behavioral parameters recorded were similar to those used for the plus maze [Shepherd et al., 1994]. The external diameter of the maze was 105 cm and the width of the lane

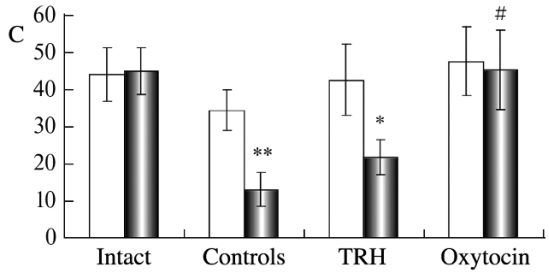

Fig. 1. Changes in anxiety levels (time spent by animals in the open arms of the EPM) after administration of OT and TRH solutions and exposure to social stress. The vertical axis shows the time spent in the open arms of the maze (sec); the horizontal axis shows groups of animals. Light columns show data for the first test and dark columns show data from the second test. *Differences between animals before and after stress, $p < 0.05$; **differences between animals before and after stress, $p < 0.005$; #differences between animals of the OT group and controls, $p < 0.005$.

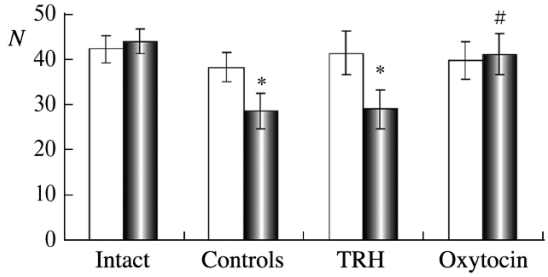

Fig. 2. Numbers of squares crossed in the EPM after administration of OT and TRH solutions and modeling of social stress. The vertical axis shows the number of squares crossed (N) and the horizontal axis shows groups of animals. Light columns show data from the first test and colored columns show data from the second test. *Differences between animals before and after stress, $p < 0.05$; #differences between animals of the OT group and controls, $p < 0.05$.

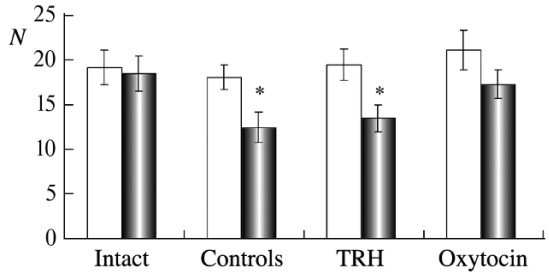

Fig. 3. Numbers of rearings performed by animals during the test in the EPM after administration of OT and TRH solutions and modeling of social stress. The vertical axis shows the number of rearings (N) and the horizontal axis shows groups of animals. Light columns show data from the first test and colored columns show data from the second test. *Differences between animals before and after stress.

of the maze was 10 cm, wall height was 27 cm, and the maze was suspended 70 cm above the floor.

Video recordings of the animals' behavior were made using a Sony DCR-HC17E PAL video camera (Japan) and a Logitech Webcam (Switzerland). Statistical processing was with the nonparametric Mann–Whitney test for independent sets and the Wilcoxon test for dependent sets, run in SPSS (PASW Statistics 18.0). Results were regarded as statistically significant at $\alpha = 0.05$.

Results. In the intact group, there were no statistically significant differences in any of the behavioral indicators measured in the first and second tests.

After social stress, animals of the control group showed a statistically significant increase in the time spent in the open arms, a decrease in the number squares crossed, and a decrease in the number of vertical rearings from baseline.

In the control group, stress was followed by a reduction in the time spent in the open arms in the EPM (p = = 0.002). Analogous changes were recorded in animals of the thyroliberin group but were not statistically significantly different from the control group (Fig. 1).

In animals of the experimental group given OT, there were no changes in anxiety 4 h after stress (p = 0.823). Animals of the control and experimental groups showed no differences in baseline anxiety levels measured in the EPM before stress ($p = 0.514$). However, in animals given OT 15 min before stress, significantly lower levels of anxiety were seen in the EPM 4 h after social stress than in animals given physiological saline ($p = 0.003$) or thyroliberin (Fig. 1).

Thus, rats given OT spent significantly longer periods of time after stress in the open arms than the control group. After stress, there were statistically significant differences in the time spent in the open arms of the maze – an indicator of the level of anxiety – between animals of the oxytocin and control groups.

Movement activity after stress decreased in the control and TRH groups as compared with the baseline level of movement activity ($p = 0.016$) (Fig. 2).

Animals of the experimental group given OT showed no statistically significant changes in movement activity ($p = 0.840$). Animals of the control and experimental groups showed no differences in terms of the level of movement activity in the EPM test before stress (p = 0.432), though statistically significant differences in this indicator between groups were seen after social stress. Animals of the group given OT showed higher levels of movement activity 4 h after the onset of stress than the groups receiving physiological saline ($p = 0.026$) and TRH (Fig. 2).

Apart from the increase in the level of anxiety and the decrease in movement activity, social stress also influenced exploratory activity in animals of the control group and the thyroliberin group. Testing in the EPM 4 h after stress decreased exploratory activity in these groups of animals, which was apparent as a reduction in the number of rearings in the control group ($p = 0.011$) and animals of the thyroliberin group ($p = 0.039$).

In the experimental group of animals given OT, the decrease in exploratory activity after stress did not reach the level of statistical significance ($p = 0.091$). The baseline level of exploratory activity showed no differences in the intact, control, and experimental groups ($p = 0.382$) (Fig. 3).

There were no statistically significant differences in the duration of grooming in any group of animals, either between the control and TRH groups, nor between the durations of grooming before and after stress.

One day after intranasal administration of solutions, all animals were tested in the elevated round maze. Comparison of data obtained on testing in the round maze identified no statistically significant between-group differences in the main parameters recorded.

Discussion. The anxiolytic properties of OT – both endogenous and exogenous – have been widely studied and confirmed by many investigators [Bartz et al., 2011; Campbell, 2010; Heinrichs and Domes, 2008; Waldherr and Neumann, 2007]. In addition, the stress-protective properties of OT may also be due to the fact that it has a role in controlling the endocrine component of the stress reaction, particularly inhibiting the activity of the hypothalamo-hypophyseal-adrenal axis [Engelmann et al., 2004; Neumann, 2002, 2008].

The present studies showed that 4 h after the beginning of social stress and 3 h after it ended, animals of the control group given physiological saline, and also the thyroliberin group, showed typical stress-induced behavioral changes such as increases in anxiety and decreases in movement and exploratory activity. At the same time, these behavioral changes were not seen in rats of the experimental group given OT. Thus, social stress was followed by detection of changes in a variety of behavioral parameters in the EPM in rats given physiological saline, while no such changes were seen in animals given OT. All these points lead to the conclusion that OT prevented the development of stress-induced behavioral changes 4 h after social stress, though testing in the elevated round maze one day later revealed no statistically significant differences between animals of any of the groups. This suggests that this type of stressor is sufficiently mild for all consequences of being placed in a group with altered social composition to have disappeared by one day.

OT receptors are found in many parts of the brain associated with the central regulation of stress and social behavior [Landgraf and Neumann, 2004]. This hormone inhibits defensive behavior and promotes increases in social interactions [Carter and Altemus, 1997; Carter et al., 2008; Donaldson and Young, 2008; Palgi et al., 2016].

OT is directly linked with the recognition of individuals of the same species, or social recognition. Mice lacking oxytocin receptors are unable to recognize their conspecifics, even after repeated presentation. Administration of OT

restores this function in these animals [Winslow and Insel, 2002]. In addition, administration of OT is known to facilitate improvements in social recognition in people with autism [Hollander et al., 2007].

TRH is a hypothalamic tripeptide [Shally, 1978] which stimulates thyrotropin synthesis and secretion in the adenohypophysis and also affects many CNS functions. TRH increases the arousal of the CNS, stimulates respiration, suppresses feeding behavior, has positive influences on depressive states, and has anticonvulsive effects [Ashmarin et al., 1989; Chepurnov et al., 2002; Jaworska-Feil et al., 2001; Knoblach and Kubek, 1994; Wan et al., 1998; Veronesi et al., 2001].

TRH is regarded as having strong antidepressant effects both in humans and in animals [Ogawa et al., 1984; Lloyd et al., 2001; Szuba et al., 2005]. Some studies have shown that TRH decreases fear and anxiety. Mice of a strain from which the gene encoding the TRH receptor has been removed show elevated levels of anxiety and depression [Zeng et al., 2007]. Administration of TRH into the cerebral ventricles decreased anxiety behavior and stress-induced increases in corticosterone levels [Gutierrez-Mariscal et al., 2008]. We have previously studied the efficacy of intranasal administration of ultralow concentrations of TRH (10^{-10} M) to decrease anxiety induced by stress due to painful electric shocks. In the three series of repeat experiments performed, we convincingly demonstrated that TRH given intranasally to male laboratory rats 15 min before exposure to the electrical pain stressor prevented the increase in anxiety without affecting the whole complex of behavior, particularly movement and exploratory activity [Vinogradova et al., 2014].

Our previous investigations identified the anxiolytic properties of TRH given intranasally 15 min before the stress stimulus – which was a weak painful electric shock [Vinogradova et al., 2014]. In contrast to control animals, rats given TRH showed no increase in anxiety in response to stress induced by painful electric shock. Changes in the parameters of movement and exploratory activity, as well as levels of emotionality, were no different from those in control animals. Thus, in the three series using painful electric shocks as stressor, TRH behaved as an anxiolytic agent, selectively acting on anxiety and not affecting the whole spectrum of changes in behavior in stress.

At the same time, on the background of exposure to the social stressor, TRH did not display its anti-anxiety properties – overall, changes in the behavior of animals given doses of this substance were no different from those in the control group.

OT is also known to have an anti-anxiety effect, though its actions are also specific. Data have been obtained showing that the overall direction of the influence of OT on behavior consists of a selective increase in susceptibility to socially significant signals [Unkelbach et al., 2008]. In this regard, the influence of OT on anxiety should be regarded as being mainly modulatory in nature, able to appear in the framework of increases in affiliation within the social group on the background of social stress – in contrast to TRH, which prevents the increase in anxiety in response to the actions of a "nonspecific" stressor and does not display its anxiolytic properties on the background of social stress. The absence of any effect of TRH on the behavioral component of the stress response after exposure to social stress suggests that the previously noted effect of this neuropeptide on the level of anxiety after stress due to painful electrical stimulation is not linked with its possible nonspecific anxiolytic action. It can be suggested that TRH is involved in activating the antinociceptive system, which leads to modulation of the level of anxiety after painful electric shock stress, though its function is not linked with changes in the state of the CNS in social interactions.

It is possible that the increase in social interactions in the framework of the "established social group" of animals given OT had less effect on their social stress as compared with control rats. Identification of the details of the influences of OT on anxiety on the background of a socially significant stressor, like the excess anxiolytic effect of TRH in "nonspecific physical" stress, will be the object of further studies.

Conclusions. The following conclusions can be made on the basis of the present data:

1. Intranasal administration of TRH 15 min before the start of social stress had no significant influence on anxiety after social stress.

2. Intranasal administration of OT solution 15 min before the start of social stress decreased stress-induced changes in behavior – an increased level of anxiety and decreased levels of movement and exploratory activity.

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