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Effects of Oxytocin on the Levels and Metabolism of Monoamines in the Brain of White Outbred Mice during Long-Term Social Isolation

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The effects of intranasal administration of oxytocin on the levels and metabolism of monoamines in symmetrical structures of the brain of white outbred mice kept under conditions of long-term social isolation were studied by HPLC. Disappearance of initial right-sided asymmetry in the content of dopamine metabolites in the striatum, increased 5-hydroxyacetic acid content in the right striatum, and disappearance of the initial left-sided asymmetry in serotonin level in the cortex were noted; we also found a decrease in norepinephrine content in the left hippocampus with appearance of asymmetry and higher content in the right olfactory tubercle. It can be hypothesized that minor changes in the serotoninergic and dopaminergic systems against the background of high reactivity of noradrenergic system represent specific response of the brain to oxytocin in aggressive animals.

Key Words: *social isolation; aggressive behavior; monoamine; hemispheric asymmetry*

Oxytocin is one of the two neuropeptides released in the pituitary gland. The classical functions of oxytocin (stimulation of labor and reflectory milk production by the mammary gland) are related to regulation of reproductive behavior in mammalian females and represent peripheral effects of the hormone [3].

Analysis of central effects of oxytocin revealed its amnestic effect in conditioned reflexes not related to parental care. It is known that oxytocin suppresses sexual activity and reduces aggression towards animals of the same species [9]. The role of oxytocin in the regulation of behavior consists in modulation of behavioral patterns, rather than regulation of particular functions of the organism [3].

It is known that monoaminergic systems of the brain take part in the formation and maintenance of behavioral patterns. Analysis of central functions of oxytocin showed that its participation in the modulation of behavior is realized via interactions with central monoaminergic systems. Thus, oxytocin promotes the formation of monogamous pairs in rodents via interaction with the dopaminergic system [9] and reduces aggression via modulation of the serotoninergic system [7].

Intranasal administration of oxytocin to humans reduced aggression and increased trust to other humans [5]. Surprising and not yet discussed feature was unilateral manifestation of these effects. For instance, oxytocin significantly attenuated left amygdala activity during exposure to both social negative emotion on faces and negative non-social scenes [10]. Moreover, oxytocin enhanced activation of the right amygdale and right striatum in healthy women during identification of the emotional state of others, but activated only the right medial frontal gyrus and the right insular cortex in female patients with depression [10]. Our previous studies showed that oxytocin also induced unilateral effects in isolated C57Bl/6 mice demonstrating aggression towards a partner kept in a group. Oxytocin increased dopamine content in the left

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cortex and serotonin content in the right hippocampus and left striatum [1]. It should be noted that isolated C57Bl/6 mice exhibited low aggression level in the resident—intruder test [1]. A question arises how oxytocin affects the state of the monoaminergic systems of the brain in highly aggressive animals.

Here we studied the effects of oxytocin on monoamine levels in the symmetric brain structures of outbred albino mice with high aggression.

MATERIALS AND METHODS

The experiments were performed on mature outbred white male mice weighing 18-22 g (Rappolovo Breeding Center).

High aggression was induced by long-term isolation in individual cages 10×10×12 cm for 10-11 weeks [2]. During resident—intruder testing, the mouse kept in a group was placed to a home cage of the isolated mouse. If the resident attacked the intruder within 4 min, the isolated mouse was considered as highly aggressive; 24 specimens were thus selected.

The effects of oxytocin on monoamine levels in the brain of mice with high aggression were analyzed 1 week after testing. To this end, oxytocin (Ellara, 5 U/ml) was administered intranasally (10 µl per nostril) to residents considered to be aggressive by the results of the test (n=13). Other animals (n=11) received the same volume of saline. Then, the resident was placed to the home cage and a mouse kept in a group was placed to this cage 5 min later. The resident was taken out of the cage and decapitated immediately after attacking.

Morphological structures were isolated from the left and right brain hemispheres on ice, weighted, and placed in different volumes of 0.01 M HCl (striatum 50 µl; hippocampus and olfactory tubercle 100 µl, and the cerebral cortex 150 µl). The samples were homogenized using UZDN-2T device and centrifuged at 15,000g for 10 min. The supernatant was collected and stored until assay at -90°C. The concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT), and their metabolites (3,4-dihydroxyphenylacetic acid, DOPAC; homovanillic acid, HVA; 5-hydroxyindoleacetic acid, 5-HIAA) were measured by reverse-phase HPLC with electrochemical detection on a Beckman Coulter chromatograph. The chromatographic system included Rheodyne 7125 injector with a 20-µl loop for injection of samples, Phenomenex column (250.0×4.6 mm) with Sphere Clone 5 u ODS(2) sorbent, and LC-4C BAS amperometric detector. The concentrations of substances were measured at 0.70 V. Mobile phase included 5.5 mM citrate-phosphate buffer with 0.7 mM octanesulfonic acid, 0.5 mM EDTA, and 8% acetonitrile (pH 3.0); elution rate 1 ml/min, the time of analysis for sample 20 min.

The obtained results were analyzed using standard GraphPad PRISM 5.0 software. Significance of between-group differences was evaluated using Student's t test. The differences were significant at $p<0.05$.

RESULTS

After placement of the intruder to the cage of the resident from the control group (administration of saline), the attack was observed in 35.0±23.1 sec. After oxytocin administration, the latency of the attack was 20.1±9.8, which did not significantly differ from the control.

In highly aggressive mice of the control group, 3 cases of asymmetric distribution of monoamines in the brain were found. They were characterized by the increased level of 5-HT in the left cortex ($p<0.05$) and reduced content of DA metabolites (HVA and DOPAC) in the left striatum ($p<0.05$; Table 1).

Analysis of the effects of oxytocin revealed a decrease in NE level in the hippocampus of the left hemisphere by 24.3% ($p<0.05$) and an increase in 5-HIAA content in the striatum of the right hemisphere by 22.7% ($p<0.05$; Table 1).

The only case when the differences between the hemispheres became significant under the effect of oxytocin was observed in the olfactory tubercle: the level of NE in the right olfactory tubercle was significantly higher ($p<0.05$; Table 1).

Interestingly, oxytocin differently affected monoaminergic systems of the various structures of the forebrain. This can be explained by heterogeneity of the compact groups of monoaminergic neurons projecting to various structures of the forebrain by their response to oxytocin. However, the interaction between monoamines and oxytocin most likely occurs in the forebrain in monoaminergic synapses. This is indirectly confirmed by the data on different density of oxytocin receptors in the brainstem and various structures of the forebrain [8].

It should be noted that the results obtained on outbred albino mice with high aggression significantly differ from the results of our study on C57Bl/6 mice [1]. First, oxytocin produced different effect on aggression: it increased the latency of the attack in isolated C57Bl/6 mice and had no effect on this parameter in outbred mice. These data coincided with the results obtained on albino rats with high aggression [4]: intranasal administration of oxytocin had no effects on the latency of attack.

When analyzing the content of monoamines in mouse brain structures, we expected that oxytocin would affect activity of the serotoninergic system. This assumption stemmed from the presence of oxytocin receptors on serotoninergic neurons [6] and from

TABLE 1. Content of Monoamines and Their Metabolites (ng/mg tissue) in Symmetric Brain Structures of Aggressive Outbred Albino Mice Subjected to Social Isolation (*M±m*)

Monoamines and their metabolites	Control group (saline)		Treatment group (oxytocin)	
	left hemisphere	right hemisphere	left hemisphere	right hemisphere
Cortex				
NE	0.1727±0.0178	0.1839±0.0152	0.1901±0.0142	0.1837±0.0113
5-HT	0.1519±0.0115	0.1249±0.0124*	0.1216±0.0167	0.1282±0.0101
5-HIAA	0.2480±0.0126	0.2326±0.0110	0.2631±0.0168	0.2626±0.0187
DA	0.02010±0.0376	0.1656±0.0288	0.2567±0.0457	0.1846±0.0167
DOPAC	0.1377±0.0196	0.1238±0.0149	0.1712±0.0230	0.1230±0.0168
HVA	0.0655±0.0035	0.0672±0.0050	0.0788±0.0065	0.0673±0.0046
Hippocampus				
NE	0.4412±0.0395	0.4320±0.0514	0.3342±0.0192+	0.3665±0.0375
5-HT	0.5565±0.0750	0.4678±0.0475	0.4645±0.0507	0.4713±0.0444
5-HIAA	0.4664±0.0322	0.4503±0.0329	0.5612±0.0365	0.5169±0.03338
DA	0.1815±0.0411	0.1710±0.0195	0.1558±0.0240	0.1767±0.0241
DOPAC	0.0821±0.0234	0.0565±0.0243	0.0964±0.0255	0.0725±0.0245
HVA	0.1432±0.0249	0.0977±0.0143	0.1227±0.0213	0.1095±0.0167
Olfactory tubercle				
NE	0.4134±0.0360	0.4788±0.0411	0.3972±0.0387	0.4819±0.0427*
5-HT	0.5520±0.0058	0.6061±0.0371	0.5222±0.0288	0.5327±0.0375
5-HIAA	0.3876±0.0258	0.4246±0.0195	0.4102±0.0177	0.44292±0.0217
DA	2.3650±0.2377	2.5110±0.2248	2.3500±0.1450	2.1470±0.1468
DOPAC	0.5845±0.0556	0.6384±0.0350	0.5876±0.0311	0.5311±0.0369
HVA	0.2004±0.0158	0.2238±0.0127	0.2193±0.0108	0.2149±0.1265
Striatum				
NE	0.2141±0.0320	0.2417±0.0303	0.2135±0.0171	0.2277±0.0207
5-HT	0.2155±0.0221	0.2179±0.0258	0.2650±0.0316	0.2693±0.0227
5-HIAA	0.4015±0.0240	0.3937±0.0155	0.4655±0.0350	0.4830±0.0303x
DA	5.4370±0.5150	6.3240±0.6650	6.120±0.8530	6.2930±0.7440
DOPAC	1.0310±0.0606	1.2800±0.0958*	1.0790±0.0864	1.1040±0.0765
HVA	0.3477±0.0213	0.3996±0.0277*	0.4112±0.0377	0.4287±0.0294

Note. $p<0.05$ in comparison with *left hemisphere (asymmetry), +left hemisphere of the control group mice, xright hemisphere of control group mice.

our previous studies on C57Bl/6 mice. However, the effects of oxytocin on the serotoninergic system of outbred albino mice were minor. Oxytocin increased 5-HIAA level in the right striatum and attenuated the initial prevalence of 5-HT in the left cortex of control mice. No other effects in the cortex were found.

Surprisingly, oxytocin produced minor effect on the DAergic system of highly aggressive mice, which consisted in disappearance of initial asymmetry of DA metabolite content in the striatum. In our previous study, this asymmetry was not observed in control C57Bl/6 mice with low aggression [1]. This fact allows suggesting that the asymmetry in DA metabolism in the striatum can be related to the high level of aggression.

A specific feature of the influence of oxytocin on the monoaminergic systems of outbred albino mice with high aggression was modulation of the norad-

renergic system. These effects were not observed in mice with low aggression [1]. In highly aggressive animals, the effects of oxytocin manifested in a significant decrease in NE level in the left hippocampus and appearance of right-sided asymmetry in the level of this neurotransmitter in the olfactory tubercle ($p<0.05$).

It can be hypothesized that the slightly pronounced changes in the state of the serotoninergic and DAergic systems associated with high reactivity of the noradrenergic system are specific features of the response of the brain of highly aggressive animals to oxytocin.

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