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EXPERIMENTAL ARTICLES

The Effects of Short-Term Stress and Long-Term Fluoxetine Treatment on the Expression of Apoptotic Proteins in the Brain

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Abstract—The effects of 2- or 8-week-long daily treatment with fluoxetine at a dose of 7.26–7.70 mg/kg given with drinking water and short-term forced-swim stress on the levels of mRNAs of anti- and pro-apoptotic proteins, that is, Bcl-xL and Bax, respectively, were studied in the brains of adult male rats using the RT-PCR method. Antiapoptotic effects of stress on the expression of these proteins were observed in the hippocampus of rats that were not treated with fluoxetine and in the midbrain after 2 weeks of the antidepressant treatment. Pro-apoptotic effects of stress were revealed in the frontal cortex of animals that were not treated with fluoxetine and after 2 weeks of fluoxetine treatment. An 8-week-long fluoxetine treatment resulted in an increase in the basal Bax expression in the hippocampus and in anti-apoptotic effects in the neocortex, which were more clearly seen after stress. The observed interaction of the effects of stress and fluoxetine on the expression of proteins of neuronal survival and plasticity may provide anti- or proapoptotic action of the antidepressant on the cells of the emotiogenic structures of the brain.

Keywords: Bcl-xL, Bax, midbrain, hippocampus, frontal cortex, fluoxetine, forced-swim test
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INTRODUCTION

Stress is one of most widespread causes of depressive disorders. Therefore, it is not surprising that antidepressants, which prevent the symptoms of this pathology, are able to affect brain mechanisms that underlie the effects of stresses on the psychoemotional condition of the body [1–3]. An important component of the pathogenic effect of stressors is induction of cell death in brain structures, primarily the hippocampus and neocortex, which are involved in the regulation of psychoemotional conditions. Cell survival and resistance or susceptibility to stress factors are determined by the ratio of expression of pro- and anti-apoptotic proteins, Bax and Bcl-xL, respectively [2]. However, the influence of the most used antidepressants in clinics, such as serotonin reuptake blockers, the most popular of which is fluoxetine, on the expression of apoptotic proteins during acute stress, as well as the possible association of this influence with the formation of the previously described behavioral and neurochemical, in particular serotonergic, antidepressant effects [4] remain unclear. The answers to these questions are important for understanding both the therapeutic and negative side effects of the drugs of this type. The aim of the present study was to examine the mRNA levels of Bax and Bcl-xL in the cortex and hippocampus, as well as in the midbrain, which is an important target of fluoxetine action [4], in animals that were subjected to a short-term forced-swim stress after 2- or 8-week-long application of the antidepressant. A 2-week-long fluoxetine administration, in contrast to a longer term, does not yet change behavior [4]; it is possible to evaluate the effects of the drug on the expression of apoptosis regulatory proteins during the therapeutic stage and the stage that precedes antidepressant administration.

MATERIALS AND METHODS

Adult male Wistar rats weighing 200–210 g at the start of the experiment were used for the study. The rats were individually housed in cages with free access to food and water. Fluoxetine was dissolved in drinking water at a concentration of 0.14 mg/mL and the animals received a daily dose of 7.26–7.70 mg/kg, which was evaluated by measurement of the volume of consumed water. The treatment continued for 0 (rats consumed pure water), 2, and 8 weeks. Half of the animals of each fluoxetine-treated and corresponding control groups was tested using the forced-swim stress. Stress exposure was performed between 10:00 and 12:00 a.m. in 46 × 20 cm glass cylinders filled with water at a temperature of 25°C to the height of 30 cm. The exposure lasted for 15 min during the first session and 5 min during the second session, which was per-

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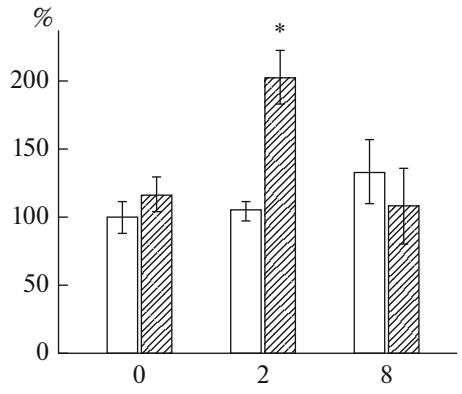

Fig. 1. The ratio between the mRNAs levels of Bcl-xL and Bax in the midbrain of adult male rats treated daily with fluoxetine at a dose of 7.5 mg/kg dissolved in drinking water for 0, 2, and 8 weeks under the basal conditions (white bars) and after stress (shaded bars). Ordinate axis, mRNA Bcl-xL/mRNA Bax ratio; abscissa axis, weeks of experiment. Data are presented as $M \pm m$ and expressed as % of the value in non-stressed rats, which did not consume fluoxetine (0 weeks), set as 100%. *$p < 0.05$ compared to the other mean values.

formed 24 h after the first one. At 1 day after the second swim session, the rats from the stressed and control subgroups were decapitated. The midbrain, frontal cortex, and hippocampus were rapidly dissected in the cold and frozen in liquid nitrogen for estimation of the mRNA level by the RT-PCR method as described previously [5]. PCR was performed with specific rat primers for Bcl-xL (forward: 5'-gtcccatcaatggcaaccat-3' and reverse: 5'-ccgccgttctcctggatccaa-3') [6] and Bax (forward: 5'-tggttgccctttctactttg-3' and reverse: 5'-gaagtaggaaaggaggccatc-3') [7], beta-actin (forward: 5'-cgtgaaaagatgacccgat-3' and reverse: 5'-attgccgatagtgatgacct-3') [8]. The PCR products were evaluated quantitatively with respect to beta-actin mRNA using scanning densitometry (BioDoc II Video Documentation System, Biometra GmbH, Germany). The parameters of PCR and detection procedures provided a linear relationship between the quantities of the original matrix and the PCR product for all of the tested mRNA. To visually compare the effects of fluoxetine and stress, the levels of transcripts and their ratios were expressed as a percentage of the corresponding index in the control animals that did not consume fluoxetine and were not subjected to stress, in which the value of the parameter was set as 100%. The effects of fluoxetine and forced-swim stress were evaluated using the two-way analysis of the variances followed by a post-hoc comparison of the means according to the Fisher LSD test. In addition, we analyzed a linear regression between the Bcl-xL mRNA/Bax mRNA ratio in the frontal cortex during stress and the duration of fluoxetine consumption. All data are presented as $M \pm m$. The differences between the means were considered as significant at $p < 0.05$.

RESULTS

The levels of Bcl-xL and Bax mRNAs did not change significantly in the midbrain after the 2- or 8-week-long fluoxetine treatment, as well as after stress exposure (data are not shown). The alterations of mRNA levels of anti- and proapoptotic proteins were opposing, although they did not reach statistical significance. However, significant effects of fluoxetine ($F(2, 33) = 3.925$, $p < 0.03$), stress ($F(1, 33) = 4.453$, $p < 0.05$) and fluoxetine × stress interaction ($F(2, 33) = 5.641$, $p < 0.01$) on the Bcl-xL mRNA/Bax mRNA ratio were observed (Fig. 1). This ratio was strongly shifted to the antiapoptotic side in the stressed animals that consumed fluoxetine for 2 weeks; this provided the statistical significance of these effects.

In the hippocampus of animals that were not treated with fluoxetine, stress increased Bcl-xL mRNA by a factor of 1.5 ($p < 0.05$). The consumption of the antidepressant for 2 or 8 weeks did not change the basal level of mRNA of the antiapoptotic protein; however, both types of fluoxetine treatment prevented the enhancing effect of stress, which was observed in the control animals. The level of mRNA of the proapoptotic protein in the hippocampus depended on both fluoxetine ($F(2, 33) = 5.532$, $p < 0.01$) and fluoxetine × stress interaction ($F(2, 33) = 3.529$, $p < 0.05$; Fig. 2). The basal level of Bax mRNA in the hippocampus of rats treated with fluoxetine for 8 weeks was significantly increased compared to other animal groups. The Bcl-xL mRNA/Bax mRNA ratio in the hippocampus of stressed animals that were not treated with fluoxetine increased compared to stressed rats that consumed the antidepressant for 2 weeks ($p < 0.05$), as well as the basal Bcl-xL mRNA/Bax mRNA ratio after 8-week exposure to fluoxetine ($p < 0.05$).

Fluoxetine treatment for 2 or 8 weeks increased the level of Bcl-xL mRNA ($F(2, 26) = 4.125$, $p < 0.03$) in the frontal cortex. In the animals that were not treated with fluoxetine the stress significantly increased Bax mRNA ($p < 0.05$) in this brain region. In the rats treated with fluoxetine for 2 or 8 weeks we did not observe any increase in the Bax mRNA level; it was substantially higher in the stressed rats without antidepressant treatment compared to the stressed rats that received fluoxetine for 8 weeks ($p < 0.05$). The Bcl-xL mRNA/Bax mRNA ratio significantly decreased in the cortex of stressed rats that were not treated with fluoxetine ($p < 0.05$; Fig. 3). There was a significant effect of fluoxetine on this ratio ($F(2, 26) = 3.609$, $p < 0.05$). The 2-week-long fluoxetine treatment increased the basal level of the ratio ($p < 0.05$), whereas the 8-week-long treatment increased both basal ($p < 0.05$) and stress-induced ($p < 0.05$) levels of the Bcl-xL mRNA/Bax mRNA ratio compared to those observed in the stressed animals that were not treated with the antidepressant.

The 2-week-long fluoxetine treatment did not result in behavioral alterations. At this time point,

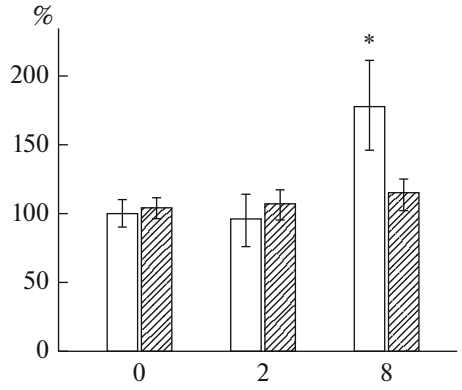

Fig. 2. The levels of Bax mRNA in the hippocampus of adult male rats treated daily with fluoxetine at a dose of 7.5 mg/kg dissolved in drinking water for 0, 2, and 8 weeks under the basal conditions (white bars) and after stress (shaded bars). Ordinate axis, Bax mRNA level; abscissa axis, weeks of experiment. Data are presented as $M \pm m$ and expressed as % of the value in non-stressed rats, which did not consume fluoxetine(0 weeks), set as 100%. $^{*}p < 0.05$ compared to the other mean values.

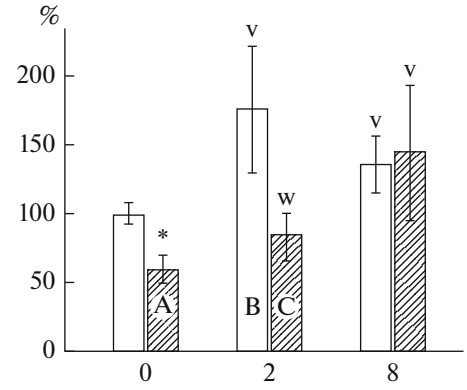

Fig. 3. The ratio between the mRNAs levels of Bcl-xL and Bax in the frontal cortex of the brain of adult male rats treated daily with fluoxetine at a dose of 7.5 mg/kg dissolved in drinking water for 0, 2, and 8 weeks under the basal conditions (white bars) and after stress (shaded bars). Ordinate axis, mRNA Bcl-xL/mRNA Bax ratio; abscissa axis, weeks of experiment. Data are presented as $M \pm m$ and expressed as % of the value in non-stressed rats, which did not consume fluoxetine (0 weeks) set as 100%. $^{*}p < 0.05$ compared to all groups escape of group C; $^{v}p < 0.05$ compared to group A; $^{w}p < 0.05$ compared to group B.

stress increased the Bax mRNA level ($F(1, 21) = 6.555$, $p < 0.02$) and, in contrast, decreased the Bcl-xL mRNA level ($F(1, 20) = 4.4259$, $p < 0.05$) and the Bcl-xL mRNA/Bax mRNA ratio ($F(1, 20) = 8.9483$, $p < 0.01$). These effects were removed after 8-week-long antidepressant treatment. There was a clear increasing effect of fluoxetine on the Bcl-xL mRNA/Bax mRNA ratio after stress exposure (Fig. 3), which was significantly linearly dependent on the duration of the antidepressant treatment [Bcl-xL mRNA/Bax mRNA] = 60.4 + 10.6[weeks of fluoxetine treatment] ($p < 0.05$).

DISCUSSION

A substantial new result of this study is the detection of the ability of fluoxetine to modify the expression of the Bcl-xL and Bax regulatory proteins of apoptosis and their response to short-term stress in important emotion-related brain structures depending on the duration of antidepressant treatment and brain structure.

The manifestation of the antidepressant effect started not from an alteration of the mRNA level of one of the pro- or antiapoptotic proteins but with a shift of their ratio mainly to the anti-apoptotic side, for example, as was observed in the midbrain and in the frontal cortex after 2 weeks of fluoxetine consumption. It is clear that the ratio of the expression of these proteins is a primary target of the regulatory action of the antidepressant because they not only play a role in cell survival [9] but also participate in neuroplasticity [10, 11] and neuroenergetic processes [12]. However, the increased basal level of Bax mRNA after 8-week antidepressant consumption is an unexpected and previously unknown effect of fluoxetine. The increased expression of this protein was observed in a model of stress-induced psychopathology [13]. Furthermore, fluoxetine can induce apoptosis in cells of peripheral tissues [14]. It is possible that this effect of fluoxetine may be responsible for the negative side effects of the drug [15].

In contrast to fluoxetine, which induced interrelated and oppositely directed alterations in the expression of pro- and antiapoptotic proteins, stress substantially shifted the expression of one of the apoptotic proteins in this pair from the normal level, which disturbed their ratio. Stress led to an increase in the mRNA level of the antiapoptotic protein in the hippocampus of rats that were not treated with the antidepressant; the increased level of this protein is associated with behavioral resilience to stress [5]. The antiapoptotic balance between mRNAs of Bcl-xL and Bax increased after 2 weeks of fluoxetine consumption in the midbrain. The proapoptotic effect of stress was observed in the cortex of control animals at the "pretherapeutic" stage of antidepressant consumption when the behavioral effect of the drug was not evident as yet; the effect was expressed in the increased Bax mRNA level and the decreased Bcl-xL level and the Bcl-xL mRNA/Bax mRNA ratio. After 8-week-long fluoxetine consumption, which caused a clear antidepressant effect on behavior and strong modification of functions of the serotonergic system of the brain [4], the ability of stress to increase the mRNA levels of both pro- antiapoptotic proteins was not found. This complex dependence of the effects of stress on the duration of antidepressant consumption may be caused by the ability of stress, on the one hand, to

damage and, on the other hand, to induce the protective mechanisms of the brain in the control animals or in rats in the initial period of drug consumption [5, 16–18]. The above-mentioned increase in the levels of proapoptotic protein in the cortex and antiapoptotic protein in the hippocampus of stressed control animals evidently reflects the dual, i.e., damaging and protective, effects of stress. During the development of the effect of fluoxetine on serotonergic neurotransmission, which was clearly seen after 8 weeks of drug consumption [4], the anti-stress effect of the drug on the expression of the apoptosis-related proteins is probably mediated by the changes in this neurochemical system of the brain. This is supported by the linear dependence of the increase in the Bcl-xL mRNA/Bax mRNA ratio on the duration of antidepressant consumption. In fact, serotonin affects neuronal firing [19] which, as was recently reported, increases the expression of the antiapoptotic Bcl-xL protein in the hippocampus [20].

CONCLUSIONS

We showed for the first time that in the midbrain, hippocampus, and frontal cortex, fluoxetine modifies the response of mRNAs of anti- and proapoptotic proteins, such as Bcl-xL and Bax, respectively, to short-term stress. The antiapoptotic effects of stress on the expression of these proteins were observed in the hippocampus of animals that were not treated with fluoxetine and in the hippocampus of rats after 2-week-long antidepressant treatment. The proapoptotic effects of stress were expressed in the frontal cortex of animals that were not treated with fluoxetine and in rats after a 2-week-long fluoxetine treatment. The 8-week-long fluoxetine treatment increased the basal expression of Bax in the hippocampus and induced the antiapoptotic effects in the cortex, which were most clearly observed in the stressed animals. The interaction of the effects of stress and fluoxetine on the expression of proteins of neuronal survival and plasticity that was revealed in the present study may mediate the pro- and anti-apoptotic effects of the antidepressant on the cells of emotion-related brain structures.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interest. The authors declared no conflict of interest.

Ethical approval. All experiments with animals were performed in accordance with international European (86/609-EEC) and Russian national guidelines for use of laboratory animals.

Informed consent. This article does not contain any studies with human participants performed by any of the authors.

REFERENCES

1. Djordjevic, A., Djordjevic, J., Elakovic, I., Adzic, M., Matic, G., and Radojcic, M.B., *Progr. Neuropsychopharmacol. Biol. Psychiatry,* 2012, vol. 36, pp. 92–100.
2. Manji, H.K., Drevets, W.C., and Charney, D.S., *Nat. Med.,* 2001, vol. 7, pp. 541–547.
3. Reus, G.Z., Abelaria, H.M., Agostinho, F.R., Ribeiro, K.F., Vitto, M.F., Luciano, T.F., Souza, C.T., and Quevedo, J., *J. Psychiatr. Res.,* 2012, vol. 46, pp. 1029–1035.
4. Shishkina, G.T., Kalinina, T.S., and Dygalo, N.N., *Neuroscience,* 2007, vol. 150, pp. 404–412.
5. Shishkina, G.T., Kalinina, T.S., Berezova, I.V., Bulygina, V.V., and Dygalo, N.N., *Behav. Brain Res.,* 2010, vol. 213, pp. 218–224.
6. Shindler, K.S., Latham, C.B., and Roth, K.A., *J. Neurosci.,* 1997, vol. 17, pp. 3112–3119.
7. Tamatani, M., Che, Y.H., Matsuzaki, H., Ogawa, S., Okado, H., Miyake, S., Mizuno, T., and Tohyama, M., *J. Biol. Chem.,* 1999, vol. 274, pp. 8531–8538.
8. Nudel, U., Zacut, M., Neuman, S., Levy, Z., and Yaffe, D., *Nucleic Acid Res.,* 1983, vol. 11, pp. 1759–1771.
9. Jonas, E.A., Porter, G.A., and Alavian, K.N., *Front. Physiol.,* 2014, vol. 5: 355.
10. Jonas, E., *Mol. Interv.,* 2006, vol. 6, pp. 208–222.
11. Li, H., Alavian, K.N., Lazrove, E., Mehta, N., Jones, A., Zhang, P., Licznerski, P., Graham, M., Uo, T., Guo, J., Rahner, C., Duman, R.S., Morrison, R.S., and Jonas, E.A., *Nat. Cell. Biol.,* 2013, vol. 15, pp. 773–785.
12. Du, J., Wang, Y., Hunter, R., Wei, Y., Blumenthal, R., Falke, C., Khairova, R., Zhou, R., Yuan, P., Machado-Vieira, R., McEwen, B.S., and Manji, H.K., *Proc. Natl. Acad. Sci. U.S.A.,* 2009, vol. 106, pp. 3543–3548.
13. Wang, X., Xie, Y., Zhang, T., Bo, S., Bai, X., Liu, H., Li, T., Liu, S., Zhou, Y., Cong, X., Wang, Z., and Liu, D., *Brain Res. Bull.,* 2016, vol. 125, pp. 134–143.
14. Zhai, F.G., Zhang, X.H., and Wang, H.L., *Clin. Exp. Pharmacol. Physiol.,* 2009, vol. 36, pp. 850–856.
15. Jakubovski, E., Varigonda, A.L., Freemantle, N., Taylor, M.J., and Bloch, M.H., *Am. J. Psychiatry,* 2016, vol. 173, pp. 174–183.
16. de Kloet, E.R., *J. Neuroendocrinol.,* 2008, vol. 20, pp. 885–892.
17. Feder, A., Nestler, E.J., and Charney, D.S., *Nat. Rev. Neurosci.,* 2009, vol. 10, pp. 446–457.
18. McEwen, B.S. *Eur. J. Pharmacol.*, 2008, vol. 583, pp. 174–185.
19. Puig, M.V. and Gener, T., *ACS Chem. Neurosci.,* 2015, vol. 6, pp. 1017–1025.
20. Lanshakov, D.A., Drozd, U.S., and Dygalo, N.N., *Biochemistry* (Moscow), 2017, vol. 82, pp. 340–344.