INTRODUCTION

An increasing body of evidence indicates that hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis and disturbing effect of glucocorticoids on neurotransmission and synaptic plasticity may be involved in the pathogenesis of depression (1-3). Prenatal stress in rats is a well-characterized animal model of depression, in which the changes exerted by elevated level of glucocorticoids are long-lasting (4). In this model, the increased immobility time in the forced swim test, disturbances in sleep and cognitive functions, decreases in sexual behavior, neurogenesis inhibition in the dentate gyrus of the hippocampus, and enhanced corticosterone concentration in light-dark cycle and after stress have been observed (5-11). Our further studies focused on elucidation of intracellular mechanism of functional regulation of glucocorticoid receptor (GR) in the prenatally stressed rats. Our previous study suggested that in prenatal stress model of depression glucocorticoid receptor (GR) function in adult rats is enhanced. However, the long-term consequences of stress, a causal factor in depression, on intracellular elements involved into the regulation of GR function is poorly examined. Mitogen-activated protein kinases (MAPKs), activity of which is disturbed in depression, are important regulators of GR action, so they can mediate the effect of stress on GR function. Therefore, the aim of the present study was to investigate the levels of active phosphorylated forms of extracellular signal-regulated kinases (ERK), Jun N-terminal kinases (JNK) and the p38 kinase in the hippocampus and frontal cortex in rats subjected to prenatal stress. The concentration of MAP kinase phosphatase (MKP-1, MKP-2) and protein phosphatase-2A (PP2A), which dephosphorylate all forms of MAP kinases, were also determined. During verification of the applied model of depression, we found that prenatally stressed rats displayed high level of immobility in the Porsolt test and that the administration of imipramine, fluoxetine, mirtazapine and tianeptine for 21 days normalized this parameter. Western blot study revealed that rats subjected to prenatal stress had decreased levels of p-JNK1 and p-JNK2 in the hippocampus and p-p38 in the frontal cortex, but the concentrations of p-ERK1 and p-ERK2 were not changed. Chronic treatment with imipramine inhibited the stress-induced decrease in p-JNK1/2, while imipramine, fluoxetine and mirtazapine blocked changes in p-p38. PP2A phosphatase level was higher in the hippocampus and frontal cortex in prenatally stressed animals than in control rats. Chronic treatment with antidepressant drugs attenuated the stress-induced increase in the level of this phosphatase, but had no effect on its concentration in control animals. There was no significant difference in MKP-1 and MKP-2 levels in both brain structures between control and prenatally stressed rats. The obtained results showed that prenatal stress decreased the levels of active form of JNK and p38, but enhanced PP2A phosphatase expression and most of these changes were reversed by antidepressant drugs. Since p-JNK and p-p38 are known to inhibit GR function their lowered levels may enhance glucocorticoid action. Furthermore, the increased PP2A concentration may intensify GR action not only by inhibition of JNK and p38 phosphorylation, but also by a direct influence on the process of GR translocation.

Key words: prenatal stress, depression, mitogen-activated protein kinases, protein phosphatases, antidepressant drugs
depression (in rats neonatally treated with clomipramine and in mice with chronically elevated corticosterone level) (14, 15).

The MAPK family is subdivided into three main classes: ERK, Jun N-terminal kinases (JNK) and the p38 kinase and they all are involved in differentiation, survival and structural and functional plasticity of neurons. So far, only ERK pathway was examined in an animal model of depression and in the brain of depressed patients. However, the levels of JNK and p38, kinases which are activated mainly by proinflammatory cytokines and environmental stresses, can be also altered in depression. Moreover, beside ERK, also JNK and p38 were shown to inhibit GR activity, in the case of JNK through direct phosphorylation of Ser-246 in GR, while p38 most probably phosphorylates one of proteins which associate with GR (16, 17). MAPKs are activated by phosphorylation of the Thr and Tyr residues within the activation loop and are inactivated by a number of different classes of protein phosphatases. Members of MAP kinase phosphatase (MKP) family are dual-specificity protein phosphatases that dephosphorylate all MAP kinases (ERK, JNK, p38) and on the other hand, synthesis of MKP-1 is enhanced by glucocorticoids (18). With regard to the depression, also important is serine/threonine protein phosphatase-2A (PP2A), which dephosphorylates all MAP kinases and also regulates serotonin uptake, synthesis of catecholamines and GR translocation and function (19-21).

Since levels of MAPKs and their phosphatases are disturbed in depression and are regulated by glucocorticoids, the aim of the present study was to investigate the levels of active, phosphorylated ERK1/2, JNK1/2 and p38 kinase and the concentration of MKP-1, MPK-2 and PP2A phosphatases in the hippocampus and frontal cortex in prenatally stressed rats (an animal model of depression).

An important point in verification of the involvement of particular neurochemical processes in an animal model of depression is its reversal or attenuation by antidepressant drugs. Surprisingly, the effect of antidepressant drugs on the level of MAP kinases and phosphatases is almost completely unexplored. Only two in vivo studies showed that amitryptyline blocked the corticosterone-induced decrease in the p-ERK1/2 concentration in the dentate gyrus of the hippocampus and fluoxetine attenuated the swimming stress-induced decrease in p-ERK2 level in the hippocampus and prefrontal cortex (15, 22). Under an in vitro condition the stimulating effect of antidepressant drugs on p-ERK2 concentration was observed only when these drugs were used at high, neurotoxic concentrations (23). In the present study, we investigate the effect of chronic treatment with various antidepressant drugs on MAP kinase and phosphatase concentrations in the hippocampus and frontal cortex of control and prenatally stressed rats. To this end, we chose imipramine, a nonselective serotonin (5-HT) and noradrenaline (NA) reuptake blocker with affinity for several neurotransmitter receptors, fluoxetine, a selective 5-HT reuptake blocker, mirtazapine, an atypical drug, which does not affect the monoamine uptake, but blocks α1-adrenergic receptors and tianeptine, a novel tricyclic drug, which contrary to other antidepressants increases neuronal uptake of 5-HT and is especially active in inhibiting the effect of stress and glucocorticoids.

Additionally, in order to verify the applied model of depression, the immobility time in the forced swim test (Porsolt’s test) was determined.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats (200-250 g), purchased from a licensed dealer, were kept under standard animal house conditions (a room temperature of 23°C, a 12/12 h light/dark cycle, the light on at 08:00), with food and water available ad libitum. A week after arrival, vaginal smears from females were collected daily in order to determine the phase of the estrous cycle. On the proestrous day they were placed with males for 12 h and next the presence of sperm in vaginal smears was check. Pregnant females were than randomly assigned to the control and stress group (n=8 in each group). All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Local Ethics Committee, Cracow, Poland.

Stress procedure

Prenatal stress was performed as previously described by Morley-Fletcher et al. (9, 10). Briefly, pregnant rats were subjected daily to three stress sessions starting at 09.00, 12.00 and 17.00 h, during which they were placed in plastic cylinders (7/12 cm) and exposed to a bright light for 45 min. Stress sessions were performed from day 14 of pregnancy until delivery. Control pregnant females were left undisturbed in their home cages. Only offspring from litters containing 10-14 pups with similar number of males and females were kept. Male offspring were selected from 21-day-old litters for the present experiment. Eight animals per group (one to 2 animals from each litter) were used for behavioral and biochemical experiments. They were housed in groups of four animals per cage under standard conditions.

Antidepressant drug administration

At 3 months of age, control (40 animals) and prenatally stressed (40 animals) male rats were injected intraperitoneally, once daily with 0.9% saline (8 animals from control and 8 animals from prenatally stressed group), imipramine hydrochloride (Pliva, Poland) (8 animals from control and 8 animals from prenatally stressed group), fluoxetine hydrochloride (Farmacom, Poland) (8 animals from control and 8 animals from prenatally stressed group), mirtazapine (Organon, the Netherlands) (8 animals from control and 8 animals from prenatally stressed group) or tianeptine (Servier, France) (8 animals from control and 8 animals from prenatally stressed group) for 3 weeks.

Antidepressant drugs were dissolved in 0.9% saline and injected at a dose of 10 mg/kg in a volume of 2 ml/kg.

Forced swimming test in rats

Animals underwent the forced swim procedure on the last 2 days of chronic treatment with antidepressant drugs i.e. about 4 months after prenatal stress.

The animals were individually subjected to two trials during which they were forced to swim in a cylinder (60 cm high, 25 cm in diameter) filled with water (25°C) up to a height of 35 cm. There was a 24-hour interval between the first and the second trial. The first trial lasted 15 min, while the second one was carried out for 5 min. The total duration of immobility was measured throughout the second trial (24).

Tissue collection

Animals were killed under non-stress conditions by rapid decapitation 24 h after the last injection with antidepressant drugs. Brains from all 80 animals were rapidly removed and the brain structures (hippocampus and frontal cortex from each rat) were dissected according to Chiu et al. (25) on ice-cold glass plates. The tissues were immediately frozen on dry ice and stored at -80°C.
Preparation of whole cell extracts

Tissues were homogenized in 5 volumes of RIPA buffer (50 mM Tris-HCl, pH=7.5) containing 150 mM NaCl, 1 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride, 1 mM sodium fluoride, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1.0% IGEPAL, 10 µg/ml of each: leupeptin, aprotinin and pepstatin. Samples were shaken in an ice-bath for 30 minutes, then they were centrifuged at 30000 × g for 20 min and supernatants were collected. Cell extracts were diluted to a protein concentration of 3 mg/ml (hippocampal) and 5 mg/ml (cerebral cortex) with lysis buffer. Protein content of lysates was determined by the method of Lowry et al. (26).

Western blotting

The cell lysates (with equal amount of protein) were mixed 1:1 with the buffer (100 mM Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.005% bromophenol blue, pH=6.8) and boiled for 5 min before loading on the gel along with molecular weight markers. Proteins were separated by SDS-PAGE (4% stacking gel, 10% resolving gel) under constant voltage (60 V in stacking gel; 120 V in resolving gel), and were transferred electrophoretically to the PVDF membrane (Boehringer Mannheim) at a 60 V constant current for 2 h. The membranes were briefly washed with the Tris-buffered saline (TBS), pH=7.5, blocked in 5% non-fat milk for 1 h at room temperature than incubated overnight at 4°C with the appropriate primary antibody. For determination of active forms of MAPK the following antibodies were used: anti-p-ERK (the mouse monoclonal antibody against phosphorylated at Tyr-202 and Tyr-204 ERK1/2 peptide fragment; sc-81492; 1:300), anti-phospho-JNK (mouse monoclonal antibody raised against a JNK sequence containing phosphorylated Thr-183 and Tyr-185; sc-6254; 1:300) and anti-phospho-p38 (rabbit polyclonal antibody against a p38 sequence containing phosphorylated Thr-180 and Tyr-182; sc-101759; 1:300). For the determination of phosphatases, we applied rabbit polyclonal antibodies: anti-MKP-1 (sc-370; 1:200), anti-MKP-2 (sc-10797; 1:200) and anti-PP2A (sc-14020; 1:300). The blots were washed: twice with TBS containing a 0.1% Tween-20 (TBST); twice with a 1% blocking solution in TBS, and were than incubated with a horseradish peroxidase-linked secondary antibody (anti-rabbit or anti-mouse respectively; 1:2000) for 1 h at a room temperature. Afterwards, the membranes were washed four times with large volumes of TBST, and immunoblots were visualized with a chemiluminescence detection kit (Boehringer Mannheim). Membranes after phospho-MAPK determination were stripped and reprobed with antibodies against unphosphorylated MAPK. Total forms of MAPK were determined with rabbit polyclonal antibodies: anti-ERK (sc-154; 1:1500), anti-JNK (sc-474; 1:1500) and anti-p38 (sc-7149; 1:1500). α-Tubulin levels were determined with mouse monoclonal antibody (sc-58667; 1:1000) and used for normalization of phosphatase bands. All used primary and secondary antibodies were from Santa Cruz Biotechnology Inc. The semiquantitative analysis of band intensity was performed using FujiLas 1000 and FujiGauge software.

Statistical analysis

The data were presented as the means±SEM and the significance of differences between the means was evaluated by the Duncan’s test following two-way analysis of variance.

RESULTS

Effects of antidepressant drugs on the immobility time in the Porsolt test

Prenatal stress significantly prolonged the immobility time in the forced swimming test, and administration of imipramine, fluoxetine, mirtazapine and tianeptine blocked this effect. The immobility time in control animals was decreased by imipramine, by not by fluoxetine, mirtazapine and tianeptine (Fig. 1).

Effects of antidepressant drugs on ERK 1 and 2 phosphorylation in the hippocampus and frontal cortex of prenatally stressed rats

Anti-phospho-ERK antibody reacted with two bands: phospho-ERK1 at 44 kDa and phospho-ERK2 at 42 kDa. P-ERK1 signals were generally weaker than p-ERK2, but were detectable in all blots, so both bands were quantified. Western

![Fig. 1. The effect of prenatal stress and antidepressant drugs on the immobility time in the Porsolt test. Control and prenatally stressed rats were injected for 21 days with vehicle, imipramine (Imi), fluoxetine (Fluo), mirtazapine (Mirt) or tianeptine (Tian). Results are expressed as the mean±SEM. *p<0.05 vs. control group; #p<0.05 vs. prenatally stressed group.](image-url)
boret analysis revealed that the concentration of phospho-ERK1 and 2 in the hippocampus and frontal cortex of rats subjected to prenatal stress did not differ from the levels of these kinases in control animals (Fig. 2A-D). Administration of imipramine, fluoxetine, mirtazapine and tianeptine for 3 weeks had no effect on these kinase concentrations in the hippocampus and frontal cortex of control and prenatally stressed rats. Total expression of unphosphorylated ERK1 and 2 also did not show any changes in all experimental groups in both brain regions (data not shown).

Effects of antidepressant drugs on JNK-1 and 2 phosphorylation in the hippocampus and frontal cortex of prenatally stressed rats

Anti-phospho-JNK antibody reacted with two bands: p-JNK1 at 46 kDa and p-JNK2 at 54 kDa and p-JNK1 signals were stronger. Prenatally stressed rats had lower p-JNK1 and p-JNK2 levels in the hippocampus than control animals (Fig. 3A, 3B). Administration of imipramine for 3 weeks attenuated the stress-induced decrease in p-JNK1 level in the hippocampus in a statistically significant manner, whereas fluoxetine, mirtazapine and tianeptine only tended to increase this parameter. All studied antidepressant drugs showed only a trend to increase the level of hippocampal p-JNK2 in prenatally stressed rats and had no effect on the concentration of p-JNK1 and p-JNK2 in control animals. There were no significant differences in the levels of p-JNK1 and p-JNK2 in the frontal cortex between control rats and animals subjected to the prenatal stress, though a decreasing tendency in stressed animals was observed (Fig. 3C, 3D). None of the investigated antidepressant drugs changed the levels of these kinases in the frontal cortex in both control and in prenatally stressed animals. Also total expression of unphosphorylated JNK1/2 did not show any changes in all experimental groups in both brain regions (data not shown).

Effects of antidepressant drugs on p38 phosphorylation in the hippocampus and frontal cortex of prenatally stressed rats

A single band for phospho-p38 at 38 kDa was observed in whole cell extracts of the hippocampus and frontal cortex. Prenatal stress decreased p-p38 level in the frontal cortex, but not in the hippocampus (Fig. 4A, 4B). Chronic treatment with imipramine, fluoxetine and mirtazapine blocked the stress-induced decrease in frontal cortex p-p38 level, while tianeptine showed only a similar but nonsignificant, tendency to change this parameter. None of antidepressant drugs modified p-p38 concentration in the frontal cortex in control animals or in the hippocampus of control and prenatally stressed rats. Total, unphosphorylated p-38 concentrations were similar in all investigated groups (data not shown).

Effects of antidepressant drugs on protein phosphatase levels in the hippocampus and frontal cortex of prenatally stressed rats

The antibodies used in our study reacted with band of 40 kDa (MKP-1), 43 kDa (MKP-2) and 36 kDa (PP2A). Amount of α-tubulin was determined simultaneously with every phosphatase and the intensities of α-tubulin bands (55 kDa) were taken as equal load controls. There was no significant difference in MKP-1 and MKP-2 concentration in the hippocampus and frontal cortex between control and prenatally stressed animals. Also total expression of unphosphorylated JNK1/2 did not show any changes in all experimental groups in both brain regions (data not shown).
stressed rats (Fig. 5A-D). Also antidepressants did not change the expression of these phosphatases.

PP2A phosphatase level was higher (by about 70% in the hippocampus and 25% in the frontal cortex) in animals subjected to prenatal stress than in control rats (Fig. 6A, 6B). Administration of imipramine, fluoxetine, mirtazapine and tianeptine for 3 weeks attenuated the stress-induced increase in the level of this phosphatase in statistically significant manner, but had no effect on its concentration in control animals.

DISCUSSION

In the present study, we applied the prenatal stress procedure as an animal model of depression, whose face, predictive and construct validity are already well documented (5-10, 27). In line with previous data, adult male rats, whose mothers were subjected to stress during the last week of pregnancy, displayed a prolonged immobility time in the Porsolt test and chronic treatment with imipramine, fluoxetine,
Mirtazapine and tianeptine inhibited the stress-induced changes in this parameter (9-11).

Biochemical analysis revealed that prenatal stress decreased the levels of p-JNK1/2 in the hippocampus and p-p38 in the frontal cortex, but had no effect on the concentration of p-ERK1/2. Among MAP kinases, the effect of JNK on the GR function is best studied. JNK was shown to phosphorylate the serine 246 in GR, and this modification is known to inhibit the activity of hormone-receptor complex on the gene transcription (12, 16). Accordingly, the selective inhibitor of JNK kinase, SP 600125, enhanced the effect of corticosterone on the reporter gene transcription in L929 cells and augmented the action of dexamethasone in hippocampal HT22 cell line (28, 29). On the other hand, JNK activation inhibited dexamethasone-induced gene transcription in HeLa cells (16). Assuming that similar mechanism is present also in vivo, the lower p-JNK level in the hippocampus of prenatally stressed rats suggests that the GR function is strengthened in these animals. Also a clinical study demonstrated lower JNK1 level in lymphocytes in depressed patients (30). In our experiments, significant reduction of p-JNK level was observed only in the hippocampus, whereas the decrease in active form of p38 kinase was found in the frontal cortex.

Fig. 5. The effect of prenatal stress and antidepressant drugs on the amount of MKP-1 in the hippocampus (A) and frontal cortex (B) and MKP-2 in the hippocampus (C) and frontal cortex (D). Control and prenatally stressed rats were injected for 21 days with vehicle, imipramine (Imi), fluoxetine (Fluo), mirtazapine (Mirt) or tianeptine (Tian). Results are expressed as the mean±SEM. Representative blots are displayed in the same order as the experimental groups in the plots below.

Fig. 6. The effect of prenatal stress and antidepressant drugs on the amount of PP2A in the hippocampus (A) and frontal cortex (B). Control and prenatally stressed rats were injected for 21 days with vehicle, imipramine (Imi), fluoxetine (Fluo), mirtazapine (Mirt) or tianeptine (Tian). Results are expressed as the mean±SEM. Representative blots are displayed in the same order as the experimental groups in the plots below. *p<0.05 vs. control group; #p<0.05 vs. prenatally stressed group.
cortex. This suggests that mechanisms controlling GR function may be tissue-dependent. Together, both observed changes, in p-JNK and p-p38, in prenatally stressed rats should lead to intensification of GR action. In contrast to JNK, only a few data are available on p38 action on GR function. They show that this kinase inhibits the effect of GR on gene transcription not by phosphorylation of GR itself, but rather by modification of some associated proteins (12, 31).

The decreased level of p-p38 in the frontal cortex was enhanced by imipramine, fluoxetine and mirtazapine, whereas the decrease in p-JNK concentration was normalized only by imipramine. These results indicate that some antidepressant drugs are able to activate the phosphorylation of JNK and p38. Such their effect was so far shown in in vitro studies mainly in relation to their anti-tumor and cytotoxic activities (32). The present results are also in line with our previous in vitro experiments, in which we found that imipramine inhibited GR-mediated gene transcription by increasing JNK activity (29).

Thus, the activation of JNK and p38 by antidepressant drugs may prevent the glucocorticoid maladaptive influence on the brain, via attenuation of GR function. Although, the activation of JNK and p38 and repression of GR function in CNS seems to be desirable in treatment of depression, however, the inhibition of GR by these kinases in some peripheral tissues may decrease also the efficacy of glucocorticoids in some chronic inflammatory diseases (31).

Since some data show a decrease in ERK1/2 level in depression, the lack of effect of prenatal stress and chronic treatment with antidepressant drugs on p-ERK concentration in the present study was unexpected. Indeed, a decrease in the ERK1/2 concentration in the human hippocampus and prefrontal cortex in post mortem study and lower level of p-ERK1/2 in different animal models of depression have been found (13-15, 33). However, there are some differences between conditions in our experiments and those applied in other models of depression, mainly regarding agents inducing depression-like symptoms and time of their action. Thus, in the study of Gourley et al. (15) mice were administered with corticosterone for 14 days, while in Qi et al. (33) rats were subjected to swimming stress for 21 days and in these study depression-inducing agents were applied in adult animals soon before determination of p-ERK. Only in the experiment described by Feng et al. (14), p-ERK level was determined, like in our experiences, long after the last depression-inducing agent administration. However, in the study of Feng et al. (14) an antidepressant drug clomipramine was administered for 14 days to neonatal Long-Evans rats, while in our experiment Sprague-Dawley rats were subjected to prenatal stress. In the model of depression applied by us alterations evoked by prenatal stress should be stable and in fact most of them are observed in adult animals. Therefore, it is surprising why we did not observe the decrease in the active form of ERK in any of the investigated brain structures. It cannot be excluded that in this particular model of the depression only other kinases from the MAPK family are decreased, such as JNK- and p38-MAPK, whose level in the above-cited investigations of other authors was not determined. Therefore, it can be assumed that in prenatally stressed animals, in which p-ERK1/2 level was unchanged, these kinases cannot be responsible for the changes in GR function, at least in the investigated brain structures.

The main finding of the present study was the observation that the level of PP2A was increased in both studied brain structures. Thus, the changes in expression of this phosphatase may be responsible not only for the decreased JNK action but also for direct regulation of the process of GR translocation from cytoplasm to nucleus (19). This phosphatase may be also involved in other changes characteristic of depression, such as decreased monoamine level or enhanced neurodegenerative processes. PP2A, a major serine/threonine phosphatase in CNS, is known to enhance serotonin transporter function which leads to decreased concentration of this neurotransmitter in synaptic cleft (20). Moreover, PP2A inhibits thyrosine hydroxylase activity, the rate-limiting enzyme in catecholamine synthesis, thus in this way it attenuates catecholamine levels (21).

Since we did not find significant changes in the level of dual-specificity protein phosphatases MKP-1 and MKP-2, the obtained results indicate that PP2A is the main phosphatase responsible for JNK and p38 dephosphorylation in prenatal stress model of depression. Also in another animal model of depression, based on neonatal clomipramine administration, the level of MKP-2 was unchanged (MKP-1 was not determined) (14). All in all, the high PP2A concentration and lack of alterations in MKP-1 and MKP-2 in the present study and increase in PP1, but not MKP-2, level in Feng et al. (14) research suggests the more important role of serine/threonine than dual-specificity protein phosphatases in depression.

Interestingly, like the changes in immobility behavior in Porsolt test, also alterations in PP2A hippocampal and frontal cortex concentration observed after the prenatal stress were normalized by all four antidepressants under investigation. The inhibitory effect of antidepressant drugs on the PP2A brain concentration point to a novel, important target of their action. By this mechanism, these drugs can regulate GR function but may also normalize several other changes observed in depression, such as the inhibition of catecholamine synthesis and decreased concentration of serotonin in synaptic cleft. Moreover, since PP2A produces neurodegenerative effects on the neurons, mainly via activation of pro-apoptotic GSK-3, attenuation of anti-apoptotic protein kinase B (PKB, Akt) and Bcl-2 dephosphorylation, antidepressant drugs, by inhibiting its expression, can prevent disturbances in cellular plasticity associated with depression (34). This assumption is supported by our previous data, which indicate that in this model of depression both dephosphorylation of GSK-3β and the level of active, pro-apoptotic form of its enzyme are increased (35). Moreover, in prenatally stressed rats the attenuation of morphological development of hippocampal neurons and inhibition of neurogenesis in dentate gyrus were observed (8, 27).

The results of the present study support hypothesis that GR function may be enhanced in depression. All three MAP kinases are known to inhibit GR function, so lower p-JNK and p-p38 brain concentration should intensify glucocorticoids action. Also the increased level of PP2A phosphatase can increase GR-mediated gene transcription not only via JNK and p38 dephosphorylation but also by the regulation of GR nucleocytoplasmic shuttling (19). Moreover, our previous study in which we found the elevated hippocampal GR level and decreased concentration of FKBP51 in the frontal cortex also supported that hypothesis (11). FKBP51 is an immunophilin, GR co-chaperone, which via reducing hormone binding affinity and nuclear translocation of GR-hormone complex inhibits GR-mediated gene transcription (36-38). The increased GR function and elevated PP2A level can in turn exacerbate hippocampal and frontal cortex damage in prenatally stressed rats.

In summary, this study showed that prenatal stress decreased the levels of the active form of JNK and p38, but enhanced PP2A phosphatase expression. These changes may be responsible for enhanced glucocorticoids action and some other disturbances observed in depression, such as, neurodegenerative changes, decreased catecholamine synthesis and serotonin concentration in synaptic cleft. On the other hand, these enzymes may be important, intracellular targets of therapeutic action of antidepressant drugs.
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Author’s address: Dr. B. Budziszewska, Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, 31-343 Krakow, 12 Smetna Street, Poland; Phone: +48 12 66 23 250; Fax: +48 12 63 74 500; E-mail: budzisz@if-pan.krakow.pl