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Prenatal stress decreases glycogen synthase kinase-3 phosphorylation in the rat frontal cortex

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Abstract:
It has been postulated that hyperactive glycogen synthase kinase-3 (GSK-3) is an important factor in the pathogenesis of depression, and that this enzyme also contributes to the mechanism of antidepressant drug action. In the present study, we investigated the effect of prenatal stress (an animal model of depression) and long-term treatment with antidepressant drugs on the concentration of GSK-3β and its main regulating protein kinase B (PKB, Akt). The concentration of GSK-3β, its inactive form (phospho-Ser9-GSK-3β), and the amounts of active (phospho-Akt) and total Akt were determined in the hippocampus and frontal cortex in rats. In order to verify our animal model of depression, immobility time in the forced swim test (Porsolt test) was also determined. We found that prenatally stressed rats display a high level of immobility in the Porsolt test and chronic treatment with imipramine, fluoxetine, mirtazapine and tianeptine normalize this change. Western blot analysis demonstrated that GSK-3β levels were significantly elevated in the frontal cortex, but not in the hippocampus, of prenatally stressed rats. The concentration of its non-active form (phospho-Ser9-GSK-3β) was decreased only in the former brain structure. No changes were found in the amounts of active (phospho-Akt) and total Akt in both studied brain structures. Chronic treatment with antidepressant drugs diminished stress-induced alterations in GSK-3β and phospho-GSK-3β levels in the frontal cortex, but had no effect on the concentration of these enzymes in the hippocampus. Moreover, levels of Akt and phospho-Akt in all experimental groups remained unchanged. Since our animal model of depression is connected with hyperactivity of the HPA axis, our results suggest that GSK-3β is an important intracellular target for maladaptive glucocorticoid action on frontal cortex neurons and in antidepressant drug effects. Furthermore, the influence of stress and antidepressant drugs on GSK-3β does not appear to impact the kinase activity of Akt.

Key words:
prenatal stress, depression, glycogen synthase kinase-3β, protein kinase B, antidepressant drugs

Introduction

Increasing evidence implicates that impaired neuronal plasticity plays a role in the pathogenesis of major depressive disorder. A reduction in the number/size of neurons and glial cells in some limbic and cortical brain regions has been observed in depressed patients [6, 28]. Moreover, several imaging studies point to abnormalities in blood flow and glucose metabolism in these brain structures [5, 18]. Disturbed cellular plasticity observed in depression is most often connected with prolonged stress and the excessive, damaging effects of glucocorticoids on the brain [35]. In fact, stress is regarded as a risk factor for depression, and a large number of depressed patients exhibit hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis [16, 22]. In experimental animals, elevated level of corticosterone and/or corticotropin releasing hormone (CRH) induce or aggravate most behavioral, biochemical and morphological depression-like changes. Thus, morphological studies suggest that chronic stress or long-term administration of glucocorticoids lead to atrophy of neurons in the hippocampus and prefrontal cortex and induce deficits in hippocampal neurogenesis [7, 27]. The exact mechanism by which glucocorticoids exert their deleterious effects on brain structures is not well understood, but it likely involves a reduction in glia number, inhibition of glutamate uptake, glucose transport and synthesis of brain-derived neurotrophic factor (BDNF) [35, 38, 39].

The majority of data on the role of intracellular processes connected with the regulation of cell survival in the pathophysiology and treatment of mood disorders come from studies investigating the mechanism of action of antidepressant and mood stabilizing drugs. These drugs are known to increase the expression of numerous plasticity-associated proteins and decrease the levels of pro-apoptotic agents such as caspase-3 or glycogen synthase kinase-3 (GSK-3) [1, 6, 15, 34]. Therefore, the potential involvement of GSK-3β in glucocorticoid-induced neuronal atrophy and depression-like action merits special attention. This enzyme is a multifunctional serine/threonine kinase that can phosphorylate and inactivate transcription factors (heat shock factor-1, CREB), promote proteolysis of β-catenin, intensify apoptosis by activation of p53 and modulate microtubule dynamics and intracellular transport [11]. The activity of GSK-3β is inhibited by phosphorylation of Ser 9, which is catalyzed predominantly by protein kinase B (PKB, Akt) [23]. The role of this kinase in mood disorders has garnered much attention since the discovery that lithium is a direct inhibitor of GSK-3β. It is also known that another mood stabilizing drug – valproate – inhibits GSK-3 activity, and some antidepressant drugs (fluoxetine and imipramine) increase its phosphorylation [15]. Moreover, evidence that GSK-3 inhibitors evoke antidepressant-like behavioral effects also supports the assumption that hyperactive GSK-3 may contribute to depression [10, 33]. However, the amount of this kinase in a prenatally stressed – animal model of depression or after a long-term treatment with antidepressant drugs remains to be investigated. Since glucocorticoids inhibit the activity of Akt and attenuate phosphorylation and inactivation of GSK-3β in peripheral tissues [36, 44], it is possible that they can also affect the level of these kinases in the brain. However, the administration of glucocorticoids to adult animals provokes transitory changes, whereas stress or glucocorticoids given during the pre- or perinatal periods lead to long-lasting disturbances in HPA axis activity, function of neurotransmitter systems and neuronal atrophy [2, 19, 43]. Furthermore, prenatal stress in rats is a well-characterized animal model of depression in which the increased immobility time in the forced swim test, disturbances in sleep and cognitive functions, decreases in sexual behavior, enhanced corticosterone concentration in light-dark cycle and after stress, the inhibition of neurogenesis and impairment of morphological development of hippocampal neurons are well documented [9, 12, 14, 20, 21, 29, 30]. These changes are long-lasting, which makes this model especially useful for studying the effects of chronic administration of antidepressant drugs. Therefore, in the present study, we investigated the effect of prenatal stress and long-term treatment with antidepressant drugs on the concentration of GSK-3β, its inactive form (phospho-Ser9-GSK-3β) and on the level of active (phospho-Akt) and total Akt in the hippocampus and frontal cortex in rats. To assess the functional output of our model, immobility time in the forced swim test (Porsolt's test) was also 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 23ºC, 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 were taken daily from females in order to determine the phase of the estrous cycle. On the proestrous day, they were placed with males for 12 h, and the presence of sperm in vaginal smears was checked. Pregnant females were then randomly assigned to the control and stress groups (n = 7 in each group). All experiments were carried out accordingly to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Local Ethics Committee, Kraków, Poland.

Stress procedure

Prenatal stress was performed as previously described by Morley-Fletcher et al. [20, 21]. 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. Twenty-one days after birth, male offspring from litters containing 10–14 pups with a comparable number of males and females were taken for experiments. Eight animals per group (one to two animals from each litter) were used for experiments. They were housed in groups of four animals per cage under standard conditions.

Antidepressant drug administration

At 3 months of age, control and prenatally stressed male rats were injected intraperitoneally, once daily with 0.9% saline, imipramine hydrochloride (Pliva, Poland), fluoxetine hydrochloride (Farmacom, Poland), mirtazapine (Organon, The Netherlands) or tianeptine (Servier, France) 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. Rats were individually subjected to two trials during which they were forced to swim in a cylinder (80 cm high, 30 cm in diameter) filled with water (25ºC) up to a height of 40 cm. There was a 24-h 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 [26].

Tissue collection

Animals were killed under non-stress conditions by rapid decapitation 24 h after the last injection with antidepressant drugs. Brains were rapidly removed, and the brain structures (hippocampus and frontal cortex) were dissected according to Chiu et al. [4] on ice-cold glass plates. The tissues were frozen on dry ice and stored at –80ºC.

Preparation of whole cell extracts

Tissues were homogenized in 5 volumes of ice-cold 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 g/ml of each: leupeptin, aprotinin and pepstatin. Samples were shaken in an ice bath for 30 min, centrifuged at 30,000 × g for 20 min, and the resulting supernatants were collected. Protein concentrations in the lysates were determined by the method of Lowry et al. [17]. Cell extracts were diluted to a protein concentration of 3 mg/ml (hippocampal) and 5 mg/ml (cerebral cortex) with lysis buffer.

Western blotting

Cell lysates (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 a 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 PVDF membranes (Boehringer Mannheim) at 60 V constant current for 2 h. The membranes were washed twice with Tris-buffered saline (TBS), pH = 7.5, blocked in 5% non-fat milk for 1 h at room temperature, then incubated overnight at 4°C with the appropriate primary antibody: anti-p-Akt 1/2/3 (Ser 473), anti-Akt, anti-p-GSK-3β (Ser 9) and anti- GSK-3β (Santa Cruz Bio-

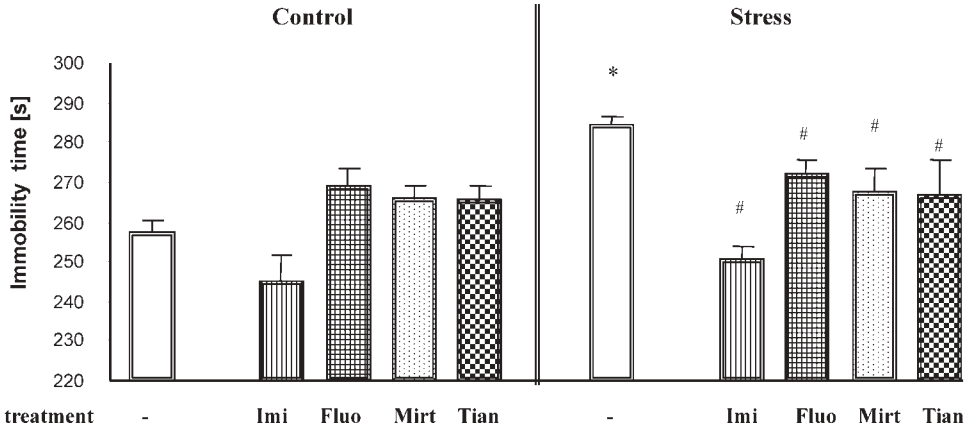

Fig. 1. The effect of prenatal stress and antidepressant drugs on the immobility time in the Porsolt test. Control and prenatally stressed rats were injected for 21 days with vehicle, imipramine (Imi), fluoxetine (Fluo), mirtazapine (Mirt) or tianeptine (Tian). Results are expressed as the mean ± SEM. * $p < 0.05$ *vs.* control group; # $p < 0.05$ *vs.* prenatally stressed group

technology, Inc.). The blots were washed twice with TBS containing a 0.1% Tween-20 (TBST), twice with a 1% blocking solution in TBS, and were then incubated with a horseradish peroxidase-linked appropriate secondary antibody (goat anti-rabbit or donkey anti-goat) 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). β-actin levels were used for normalization. The semiquantitative analysis of band intensity was performed using FujiLas 1000 and FujiGauge software.

Statistical analysis

The data were presented as the means ± SEM. Statistical comparisons were performed with the use of two-way analysis of variance (ANOVA) with group (control *vs.* prenatal stress) and treatment (vehicle *vs.* antidepressant drugs) as factors, followed by Duncan's test. In all cases, $p < 0.05$ was considered statistically significant.

Results

Effects of antidepressant drugs on immobility time in the Porsolt test

Prenatal stress significantly prolonged the immobility time in the forced swim test (Fig. 1). ANOVA indicated a significant group (control *vs.* prenatal stress) effect ($F_{1,70} = 8.01$, $p < 0.05$) and significant group x treatment interaction ($F_{4,70} = 4.02$, $p < 0.05$). A *post-hoc* Duncan's test showed that imipramine, fluoxetine, mirtazapine and tianeptine did not change the immobility time in control animals ($p > 0.05$) but decreased this parameter in rats subjected to prenatal stress ($p < 0.05$).

Effects of prenatal stress and antidepressant drugs on GSK-3β and phospho-GSK-3β concentration in the frontal cortex and hippocampus

Bands of GSK-3β and phospho-GSK-3β (Ser 9), determined in whole cell extracts of the frontal cortex and hippocampus, were observed at 47 kDa in western blot analysis (Fig. 2 and 3). For GSK-3β levels in the frontal cortex, ANOVA indicated a significant group (control *vs.* prenatal stress) effect ($F_{1,70} = 10.28$, $p < 0.05$) and significant group x treatment interaction ($F_{4,70} = 3.10$, $p < 0.05$). Specifically, prenatally stressed rats had higher GSK-3β concentrations in the frontal cortex compared to control animals ($p < 0.05$) (Fig. 2A). Administration of imipramine, fluoxetine, mirtazapine and tianeptine for 3 weeks did not change GSK-3β levels in control animals, although the increase in kinase levels induced by prenatal stress were attenuated by imipramine, fluoxetine and mirtazapine ($p < 0.05$). Chronic treatment with tianeptine tended to decrease GSK-3β level in prenatally stressed rats, but this change did not reach statistical significance ($p = 0.051$). Since GSK-3β activity is negatively regulated by phosphorylation on Ser-9, the level of phospho-GSK-3β was also determined in the same sample. For phospho-GSK-3β levels in the frontal cortex, ANOVA indicated a significant treatment effect ($F_{4,70} = 3.26$, $p < 0.05$) and significant group x treatment interaction ($F_{4,70} = 3.71$, $p < 0.05$). The phospho-GSK-3β concentration was lower in rats subjected to prenatal stress than in control animals ($p < 0.05$) (Fig. 2B). None of the anti-

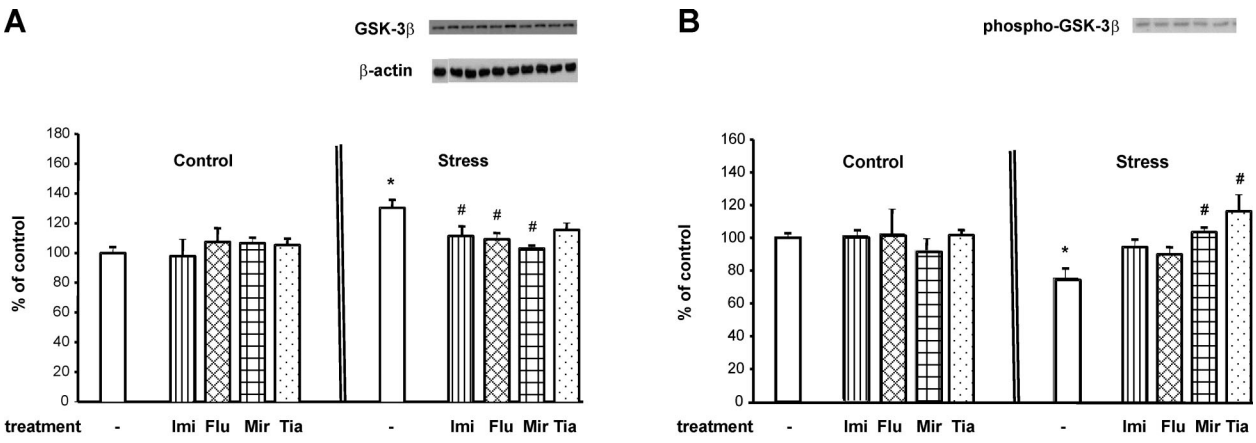

Fig. 2. The effect of prenatal stress and antidepressant drugs on the amount of GSK-3β (**A**) and phospho-Ser9-GSK-3β (**B**) in the frontal cortex. Control and prenatally stressed rats were injected for 21 days with vehicle, imipramine (Imi), fluoxetine (Fluo), mirtazapine (Mirt) or tianeptine (Tian). Results are expressed as a percentage (SEM) of no-stress/vehicle group. *$p < 0.05$ *vs.* control group; #$p < 0.05$ *vs.* prenatally stressed group. Representative blots are displayed in the same order as the experimental groups in the plots below

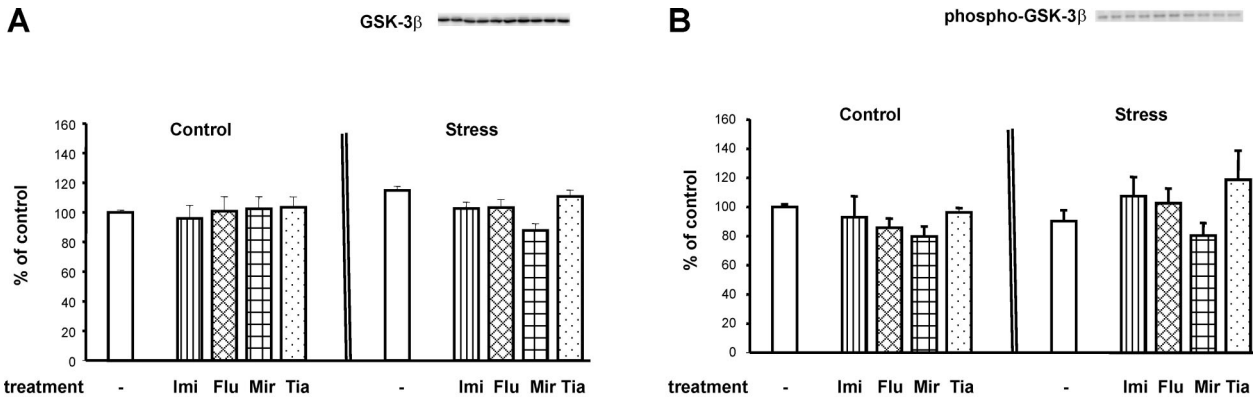

Fig. 3. The effect of prenatal stress and antidepressant drugs on the amount of GSK-3β (**A**) and phospho- Ser9-GSK-3β (**B**) in the hippocampus. Control and prenatally stressed rats were injected for 21 days with vehicle, imipramine (Imi), fluoxetine (Fluo), mirtazapine (Mirt) or tianeptine (Tian). Results are expressed as a percentage (± SEM) of no-stress/vehicle group. Representative blots are displayed in the same order as the experimental groups in the plots below

depressant drugs used in this study changed phospho-GSK-3β levels in control animals. Chronic treatment with mirtazapine and tianeptine attenuated the prenatal stress-induced decrease in phospho-GSK-3β in a statistically significant manner ($p < 0.05$), while imipramine and fluoxetine showed only a tendency to normalize this parameter ($p = 0.06$ and $p = 0.09$, respectively).

Unlike the frontal cortex, there was no significant group effect ($F_{1,70} = 0.85$, $p > 0.05$) or significant group x treatment interaction ($F_{4,70} = 1.98$, $p > 0.05$) in hippocampal GSK-3β levels. There was also no significant difference in the phospho-GSK-3β concentration in the hippocampus between control rats and animals subjected to prenatal stress (group effect: $F_{1,70} = 1.10$, $p > 0.05$; group x treatment interaction: $F_{4,70} = 0.58$, $p > 0.05$). However, an increasing tendency in hippocampal GSK-3β concentration in stressed animals was observed ($p = 0.12$) (Fig. 3A, B). None of the investigated antidepressant drugs changed

Tab. 1. The effect of prenatal stress and antidepressant drugs on the amount of Akt and phospho-Akt (p-Akt) in the hippocampus and frontal cortex

Treatment	Frontal cortex Akt	Frontal cortex p-Akt	Hippocampus Akt	Hippocampus p-Akt
Vehicle	100.0 ± 6.9	100.0 ± 9.5	100.0 ± 1.6	100.0 ± 5.1
Imipramine	107.9 ± 6.1	157.2 ± 26.3	102.2 ± 5.8	82.4 ± 15.6
Fluoxetine	111.6 ± 5.2	101.5 ± 12.3	104.5 ± 5.4	93.5 ± 15.4
Mirtazapine	112.8 ± 3.4	97.2 ± 7.6	106.1 ± 11.1	94.8 ± 11.7
Tianeptine	101.6 ± 3.8	97.8 ± 6.3	109.6 ± 8.4	93.5 ± 6.0
Stress/vehicle	118.8 ± 4.6	114.0 ± 5.0	118.7 ± 10.0	116.7 ± 12.3
Stress/imipramine	124.5 ± 10.9	95.7 ± 12.2	93.7 ± 6.8	102.2 ± 24.5
Stress/fluoxetine	118.9 ± 15.7	103.6 ± 12.6	97.4 ± 5.7	109.9 ± 22.6
Stress/mirtazapine	123.2 ± 11.1	116.2 ± 25.2	103.6 ± 6.5	131.5 ± 15.3
Stress/tianeptine	86.9 ± 6.2	104.3 ± 11.5	116.4 ± 7.2	90.6 ± 11.7

Control and prenatally stressed rats were injected for 21 days with vehicle, imipramine, fluoxetine, mirtazapine or tianeptine. Results are expressed as a percentage (± SEM) of no-stress/vehicle group.

GSK-3 and phospho-GSK-3β concentrations in the hippocampus in either the control or prenatally stressed animals.

Effects of prenatal stress and antidepressant drugs on Akt and phospho-Akt concentrations in the frontal cortex and hippocampus

To examine the regulation of GSK-3β levels by Akt, the concentrations of Akt and its active form phospho-Akt were determined in whole cell extracts from the hippocampus and frontal cortex. Bands of Akt and phospho-Akt (Ser-473) were observed at 60 kDa. Western blot analysis revealed that concentrations of Akt and phospho-Akt in rats subjected to prenatal stress did not demonstrate differences as compared to control animals (Tab. 1). ANOVA indicated no significant group effect ($F_{1,70} = 0.07$, $p > 0.05$) or significant group x treatment interaction ($F_{4,70} = 0.78$, $p > 0.05$) in hippocampal p-Akt levels or in the concentration of Akt (group effect: $F_{1,70} = 0.76$, $p > 0.05$; group x treatment interaction: $F_{4,70} = 0.26$, $p > 0.05$). There was also no significant difference in frontal cortex Akt levels (group effect: $F_{1,70} = 0.18$, $p > 0.05$; group x treatment interaction: $F_{4,70} = 0.42$, $p > 0.05$), whereas in Akt concentration, there was a significant group x treatment interaction ($F_{4,70} = 3.09$, $p < 0.05$), but no group effect ($F_{1,70} = 0.63$, $p > 0.05$). No significant increase in active or total Akt levels was observed in tissues of stressed animals, which showed only a 14–19% change. The administration of imipramine, fluoxetine, mirtazapine and tianeptine for 3 weeks caused no change in Akt and phospho-Akt concentrations in the hippocampus and frontal cortex of control and prenatally stressed rats. However, imipramine showed a strong tendency to increase the level of phospho-Akt in the frontal cortex in control animals, whereas mirtazapine tended to increase the concentration of this kinase in the hippocampus in prenatally stressed rats.

Discussion

Prenatal restrain stress in rats was used in the present study as an animal model of depression. The predictive and construct validity of this model was already reported [12, 14, 20, 21, 29, 30]. In accordance with previous results, the present study showed that prenatally stressed rats displayed a prolonged immobility time in the Porsolt test. Moreover, in line with our previous data, all antidepressant drugs under study inhibited the stress-induced increase in immobility time, i.e., they showed antidepressant-like behavior [41].

The main finding of the present study is the observation that rats subjected to prenatal stress had increased amounts of GSK-3β in the frontal cortex. The promoter region of the GSK-3β gene possesses

a number of putative transcription factor binding sites, but does not have a *glucocorticoid response element* sequence. Thus, a direct effect of glucocorticoids on the transcription of this gene is rather unlikely [13]. We found decreased concentrations of pSer9-GSK-3β in the frontal cortex of prenatally stressed animals, which indicated that the increase in GSK-3β can result from attenuation of Ser 9 phosphorylation and in consequence can lead to a disturbance in the proportion of these two enzyme forms.

Among several regulators of GSK-3β, PKB/Akt is considered to be the principal enzyme that catalyzes phosphorylation of Ser 9 and subsequently inhibits its activity. However, we did not observe a decrease in the level of active (phospho-Akt) and total Akt in either of the studied brain structures. Therefore, our results indicate that attenuated GSK-3β phosphorylation is not evoked by enhanced levels/activity of Akt. In addition to Akt, other protein kinases such as protein kinase A and protein kinase C have also been shown to phosphorylate GSK-3β, while dephosphorylation of Ser 9 is catalyzed by protein phosphatase 1 and protein phosphatase 2A [23]. Interestingly, our unpublished data indicate that in prenatally stressed rats, the level of protein phosphatase 2A was increased in the frontal cortex. In another animal model of depression, based on neonatal clomipramine administration, the concentration of protein phosphatase 1 in the frontal cortex and hippocampus was elevated [8]. This finding suggested that enhanced dephosphorylation, rather than disturbance in Ser 9 phosphorylation of GSK-3β, is responsible for the changes in the level of this enzyme in prenatally stressed rats.

Alterations in GSK-3β phosphorylation can be connected with excessive glucocorticoid action during the prenatal phase. It is well known that stress or glucocorticoids given in the pre- or perinatal period lead to long-lasting changes, such as disturbances in neurotransmitter function, neuronal plasticity and hyperactivity of the HPA axis. We and other authors observed that in this model of depression, the corticosterone level was increased at the end of the light phase and 1 h following acute stress [12, 41, 42]. Therefore, it is not possible to say whether changes in GSK-3β phosphorylation are already connected with increased corticosterone levels in the prenatal period or with an elevated concentration of this hormone in adult animals. Moreover, in this model of depression, glucocorticoid receptor (GR) function seems to be intensified. In the previous study, we found that prenatally stressed rats had a decreased concentration of FKBP51, an immunophilin that decreases GR function, in the frontal cortex [41]. Thus, elevated corticosterone levels and/or increased GR action may enhance synthesis of some pro-apoptotic factors, such as GSK-3β, and induce/intensify apoptosis. In line with our data, an *in vitro* study showed that glucocorticoids increase levels of the activated form of GSK-3β in neuronal cell culture [40].

The deleterious effects of chronic stress or glucocorticoid administration on the structure and function of neurons thus far have been examined mainly in the hippocampus. However, several studies have demonstrated that the frontal cortex is also a key target of the maladaptive action of glucocorticoids [3]. Indeed, we found changes in GSK-3β/phospho-GSK-3β proportions in the frontal cortex of prenatally stressed rats but not in the hippocampus. Similarly, in a previous study, the alterations in FKBP51 concentration were observed only in the frontal cortex. These data further suggest that the frontal cortex is vulnerable to the deleterious effects of glucocorticoids. However, contrary to our results, an animal model of depression using chronic mild stress found enhanced GSK-3β levels in the hippocampus (in the cortex was not investigated) [37]. This indicates that the changes in hippocampal GSK-3β concentration depend on the model of depression and period of exposure to stress (prenatal *vs*. adult animals).

Prenatal stress-induced increases in active GSK-3β levels in the frontal cortex were normalized by long-term treatment with imipramine, fluoxetine and mirtazapine, whereas tianeptine showed only a tendency to decrease this parameter. The non-active form of GSK-3β, pSer9-GSK-3β, was increased by mirtazapine and tianeptine, whereas imipramine and fluoxetine only tended to enhance the content of this kinase form. On the other hand, we did not observe any changes in the level of GSK-3β or phospho-Ser9-GSK-3β in control animals after chronic treatment with imipramine, fluoxetine, mirtazapine or tianeptine. So far, only imipramine and fluoxetine, given once at relatively high doses, have been demonstrated to increase phospho-Ser9-GSK-3β concentrations in the mouse prefrontal cortex [15]. The differences between our and the above-cited studies likely result from various experimental conditions such as time and dose of antidepressant administration and different animal species. Interestingly, Roh et al. [32] found that imipramine is capable of blocking hypoxia-

induced serine-dephosphorylation of GSK-3β. This finding, like our results, indicates that antidepressant drugs normalize GSK-3β phosphorylation disturbed by various agents (stress, hypoxia). In addition to GSK-3β, antidepressant drugs are known to inhibit many effects exerted by glucocorticoids, and interestingly, inhibition of glucocorticoid synthesis intensifies their action [24, 31].

In line with our observations on GSK-3β phosphorylation, chronic treatment with antidepressant drugs had no statistically significant effects on the amount of active and total Akt in both studied tissues in control animals. However, imipramine tended to increase phospho-Akt levels in the frontal cortex in control animals. There is little available data concerning the effect of imipramine on Akt, but at least one study demonstrated that long-term treatment with imipramine enhanced phospho-Akt levels in several brain regions [25]. However, in that report, imipramine was used at a higher dose than in our study and the experiment was performed in mice.

In conclusion, prenatal stress increased the concentration of the active form of GSK-3β in the rat frontal cortex, and this alteration may be caused, at least in part, by the alteration in the dynamics of phosphorylation/dephosphorylation of this enzyme. Antidepressants representing various chemical groups and pharmacological mechanisms of action attenuated the stress-induced changes in GSK-3β tissue content, but with varying potency. The obtained results suggest that GSK-3β can be an important intracellular target in the maladaptive action of glucocorticoids on the frontal cortex neurons and in antidepressant drug effects. Furthermore, the influence of stress and antidepressant drugs on the GSK-3β does not seem to involve the kinase activity of Akt.

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