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Fluoxetine affects antioxidant system and promotes apoptotic signaling in Wistar rat liver

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRI) are a treatment of choice for stress related disorders including clinical depression and a range of anxiety-related disorders. In the experimental animals, chronic stress paradigms are considered as a model of depression, and in that context are used for examining the effects of different drug treatments. The present research was designed to investigate the effect of SSRI fluoxetine on antioxidant status and apoptotic signaling in Wistar rat liver, which is a central organ for activation and detoxification of many xenobiotics and reactive oxygen species. We also investigated whether chronic fluoxetine treatment exhibits the same effects in the liver of control animals vs. animals stressed by chronic psychosocial isolation. Our results revealed that fluoxetine downregulated the activity of superoxide dismutases and upregulated the activity of glutathione peroxidase in both rat groups, while elevating glutathione reductase activity and total antioxidant status only in stressed animals. These results suggested that fluoxetine interfered with stress-induced pathways of oxidative defense in the liver. In addition, in both experimental groups, fluoxetine induced several hallmarks of apoptosis in the liver, including a decrease in Bcl-2 expression and increased DNA fragmentation. However, apoptotic alterations were more pronounced in stressed animals, suggesting that stress related oxidative damage could have primed apoptotic effects of fluoxetine.

1. Introduction

Fluoxetine belongs to the selective serotonin reuptake inhibitor group of antidepressants, which effectively alleviate symptoms of a wide spectrum of mood disorders (Wong et al., 1995). The central organ for its activation is the liver, where fluoxetine undergoes extensive metabolic conversion, leading to the formation of the active metabolite norfluoxetine among multiple other metabolites (Altamura et al., 1994). Due to inhibition of its own metabolism, elimination of fluoxetine and norfluoxetine from the body is extremely slow (Crewe et al., 1992). When fluoxetine is administered intraperitoneally, the drug rapidly reaches high concentrations in the liver. Fluoxetine and norfluoxetine were found to exert potentially toxic multiple effects on energy metabolism in rat liver mitochondria (Souza et al., 1994). This seems to be a consequence of the solubilization of the drug and/or its metabolites in the inner mitochondrial membrane. However, the molecular basis of fluoxetine-induced hepatotoxicity (Cai et al., 1999; Johnston and Wheeler, 1997; Friedenberg and Rothstein, 1996) is not well understood as yet. There is also scarce information on the influence of fluoxetine on antioxidant enzymes. While some reports suggest that fluoxetine restores antioxidant capacity in rat brain, as well as in the liver (Zafir and Banu, 2007; Bilici et al., 2001), others claim that the therapy does not significantly alter these parameters in depressed patients (Galecki et al., 2009).

Numerous studies have confirmed that mood disorders are characterized by the activation of immune and inflammatory systems (Sluzewska et al., 1996; Leonard, 2001), both on the periphery and in the central nervous system, and that activation of these systems favor the production of reactive oxygen species by various mechanisms (Fialkow et al., 2007). Oxidative stress is defined as a condition arising from a disproportion between the generation of reactive oxygen species (e.g. superoxide anion, hydrogen peroxide and hydroxyl radicals), and the activity of antioxidant defense systems: superoxide dismutases (SOD) that include copper/zinc superoxide dismutase (CuZnSOD) and manganese superoxide dismutase (MnSOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), as well as, nonenzymatic antioxidants (Kohen and Nyska, 2002). Cells appear capable of handling low doses of oxidant stressors/reactive metabolites, while higher doses overwhelm their protective capacity and lead to damage or cell death. Thus, it has been shown that oxidative stress can activate the mitochondrial pathway of apoptosis through upregulation of Bax and downregulation of Bcl-2 (Herrera et al., 2001).

In experimental animals, chronic stress paradigms are considered as models of depression (Willner, 1997), and are widely used for examining the effects of treatments with different drugs. Our recent study (Djordjevic et al., 2010) showed compromised antioxidant defense in

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the liver under chronic stress. This finding has to be taken into account if any side effects of drug treatment, potentially aggravating normal liver functions, are to be avoided. In the present study we hypothesize that chronic fluoxetine administration affects antioxidant status and apoptotic signaling in the context of chronic psychosocial stress-evoked liver aberrations, and we aim to deduce possible association of fluoxetine action with oxidative damage and apoptosis under chronic stress conditions.

2. Materials and methods

2.1. Materials

A fluoxetine-hydrochloride reference standard was kindly gifted by Galenika a.d., Belgrade, and Flunirin® capsules (containing 20 mg of fluoxetine-hydrochloride) were purchased from the same company. Commercial kits from Randox Laboratories were used for activity assays and total antioxidant status, and all measurements were performed on automated Randox RX Daytona Chemistry Analyzer (Crumlin, UK). Polyvinylidene difluoride (PVDF) membrane (Immobilon-P membrane) was obtained from Millipore Corporation (USA) and enhanced chemiluminescence (ECL) reagent pack containing Rabbit IgG, HRP-linked whole antibody and ECL Mouse IgG, HRP-linked whole antibody were obtained from Amersham Pharmacia Biotech, UK. Rabbit polyclonal anti-β-actin (ab8227) was obtained from Abcam (USA), while mouse monoclonal antibodies anti-Bcl2 and anti-Bax were obtained from Santa Cruz Biotechnology (USA).

2.2. Preparation of fluoxetine-hydrochloride solution

The capsules of Flunirin® were used to obtain purified fluoxetine. The capsules were emptied and dissolved in distilled, sterile water with the aid of ultrasound bath, and the solution was filtered through Whatman No. 42 filter paper. The concentration of fluoxetine-hydrochloride in the purified preparation was determined by colorimetric method essentially as described by Prabhakar et al. (1999), using pure fluoxetine hydrochloride (Elli Lilly, Reference Standard Lot 00IPD5, p = 100%, v = 0.07%) as the standard. Fluoxetine-hydrochloride preparation was diluted with distilled water to the final concentracion of 5 mg/ml and was administered intraperitoneally at a daily dose of 5 mg/kg body mass.

2.3. Animal care and treatment

All experiments were performed in adult (2.5 months old) Wistar male rats (body mass 330–400 g), housed four per standard size cage and offered food (commercial rat pellets) and water *ad libitum*. Light was kept on, between 07:00 am and 07:00 pm, and room temperature was kept at 20 ± 2 °C. All animal procedures were approved by the Ethical Committee for the Use of Laboratory Animals of the VINCA Institute of Nuclear Sciences, according to the guidelines of the EU registered Serbian Laboratory Animal Science Association (SLASA). For the purpose of the experiment, animals were divided in four groups, housed in groups of four per cage (control + vehicle and control + fluoxetine groups) or individually (stress + vehicle and stress + fluoxetine groups). Individual housing was used as a model of chronic social isolation stress during which animals had partial auditory and olfactory experiences, but were deprived of any visual or tactile contact with other rats. Fluoxetine-hydrochloride dissolved in distilled water was administered intraperitoneally at 09:00 h during a 21-day period, at a daily dose of 5 mg/kg body mass. Drug dose was chosen based on previous reports (Elaković et al., 2010; Detke et al., 1997). Long-term fluoxetine treatment was applied in control + fluoxetine group as it stands, and in animals previously exposed to 21-days social isolation (stress + fluoxetine group). Both vehicle groups received distilled water under the same conditions as matching fluoxetine treated groups. All the experiments were replicated two times, each time with the new group of eight animals per group, and measurements of each sample were repeated 3 times.

2.4. Isolation of tissue and preparation of whole cell extract

After sacrifice, livers of animals from each group were perfused *in situ*, carefully excised and kept frozen (−70 °C) until further analyses. After a swift thawing, livers were weighed and homogenized at 4 °C by 20 strokes of a Potter-Elvehjem homogenizer (1:4 = tissue mass: vol) in 20 mM HEPES pH 7.4 buffer (containing 1 mM Na_2EDTA, 10% glycerol, 150 mM NaCl, 20 mM Na_2MoO_4, 0.15 mM spermidine, 0.1 mM PMSF, 5 μg/ml antipain, 5 μg/ml leupeptin, 5 μg/ml aprotinin and phospatase inhibitors: 20 mM β-glycerophosphate, 5 mM $Na_4P_2O_7 \times 10H_2O$, 2 mM Na_3VO_4, 25 mM NaF and 0.5% Triton X-100). Homogenates were incubated on ice for 2 h (with frequent vortexing) in the same buffer and then centrifuged at 12,000 rpm for 30 min at 4 °C to obtain supernatant which was used as a whole cell extract.

2.5. Liver antioxidant enzymatic activities and total antioxidant status

Total SOD activity was determined using a commercial kit (SD125, Randox Laboratories, Crumlin, UK). Briefly, this method uses xantine and xantine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. One unit of SOD activity causes a 50% inhibition of the rate of reduction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride. The SOD activity was expressed as unit per mg of protein. Catalase activity was determined by the method of Claiborne (1985), using H_2O_2 as substrate. The disappearance of H_2O_2 was followed spectrophotometrically at 240 nm. Catalase activity was also expressed as units per mg of protein. The activity of glutathione peroxidase was assayed by using a commercial kit (RS504, Randox Laboratories, Crumlin, UK), and the activity was expressed as units per mg of protein. Glutathione reductase activity was also measured by a commercial kit (GR2368, Randox Laboratories, Crumlin, UK) following the oxidation of NADPH to $NADP^+$ during the reduction of oxidized glutathione and the activity was expressed as units per g of protein. Total antioxidant status (TAS) was evaluated using the Randox kit (NX2332, Randox Laboratories, Crumlin, UK). In the TAS assay 2,2′-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^{\bullet+}$. Its relatively stable blue-green color was measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree that is proportional to their concentration. All measurements were performed on automated Randox RX Daytona Chemistry Analyser (Crumlin, UK).

2.6. Western blot detection of Bax and Bcl-2 protein

The protein concentrations in the samples were analysed by the method of Markwell et al. (1978). The samples were mixed with denaturing buffer according to Laemmli (1970) boiled for 5 min at 100 °C, and 60 μg of protein was subjected to electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). After electrophoresis, proteins were transferred onto PVDF membrane (Immobilon-P membrane, Millipore) using a blot system (Transblot, BioRad). Membranes were incubated with respective primary and secondary antibodies, signal was developed using enhanced chemiluminescence reagent (ECL, Pierce) and the membranes were exposed to X-ray film. Protein molecular mass standards (PageRuler™ Plus Prestained Protein Ladder, Fermentas) were used for calibration. Mouse monoclonal antibodies anti-Bcl2 and anti-Bax (Santa Cruz Biotechnology) were used to detect these proteins, while rabbit polyclonal anti-β-actin (ab8227, Abcam) antibody was used to detect actin as a loading control. Blots were developed with ECL Rabbit IgG, HRP-

linked whole antibody and ECL Mouse IgG, HRP-linked whole antibody (Amersham). Densitometry of protein bands on X-ray film was performed by Image J analysis PC software.

2.7. DNA fragmentation assay

Frozen samples of liver tissue were homogenized in lysis buffer containing 5 mM Tris–HCl pH 8.0, 20 mM Na_2EDTA and 0.5% Triton X-100. Homogenates were centrifuged at 27,000×*g* for 20 min to separate intact chromatin in the pellets from fragmented DNA in the supernatant fractions. The fragmented DNA in the supernatants were digested with 100 ng/ml ribonuclease and 20 ng/ml protease, purified by the phenol/chloroform extraction method and precipitated with ethanol/ammonium acetate. The DNA was electrophoresed on 1.6% agarose gel containing ethidium bromide (Bagchi et al., 1998).

2.8. Statistical analysis

Data are presented as mean ± S.E.M. from 3 independent measurements of samples obtained from 2 separate groups of eight animals. To determine the effects of stress and fluoxetine treatment, as well as their interaction, we used a two-way ANOVA test followed by the *post hoc* Tukey test. All statistically significant differences between the groups are given as $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$, as presented in the Figures, while the effects of the stress and fluoxetine factors and their interaction are presented in the Section 3.

3. Results

3.1. Activity of antioxidant enzymes and total antioxidant status

Analyzing the activity of antioxidant enzymes, two-way ANOVA revealed a significant main effect of fluoxetine treatment [$F(1,20)=33.48$, $P<0.001$] on SOD activity. We detected a decrease in SOD activity in the liver of both control and stressed animals treated with fluoxetine ($^{*}P<0.05$) with respect to the untreated group, and in the stressed group treated with the fluoxetine compared to the untreated stressed group ($^{\dagger}P<0.05$) (Fig. 1A). The catalase activity did not show any significant alterations upon stress and/or fluoxetine treatmant (Fig. 1B). A significant main effect of fluoxetine treatment [$F(1,18)=65.75$, $P<0.001$] was also detected for GPx activity. GPx activity was increased in both groups treated with fluoxetine with respect to the untreated group ($^{**}P<0.01$), and in stressed group treated with the fluoxetine with respect to the untreated stressed group ($^{\dagger\dagger}P<0.01$) (Fig. 1C).

Two-way ANOVA revealed a significant main effect of chronic stress [$F(1,20)=12.07$, $P<0.01$], and fluoxetine treatment [$F(1,20)=12.26$, $P<0.01$], as well as a significant interaction between these factors on the glutathione reductase activity [$F(1,20)=9.83$, $P<0.01$]. Its activity was significantly increased by fluoxetine in the stressed group with respect to all other groups ($^{***}P<0.001$, $^{\dagger\dagger\dagger}P<0.001$, $^{\#\#\#}P<0.001$) (Fig. 1D). Regarding total antioxidant status, two-way ANOVA showed main effect for chronic stress [$F(1,16)=98.60$, $P<0.001$], for fluoxetine treatment [$F(1,16)=181.80$, $P<0.001$], and a significant main effect for interaction between chronic stress and fluoxetine treatment [$F(1,16)=45.20$, $P<0.001$]. TAS was increased in the stressed group treated with fluoxetine in comparison to all other groups ($^{***}P<0.001$, $^{\dagger\dagger\dagger}P<0.001$, $^{\#\#\#}P<0.001$) (Fig. 1E).

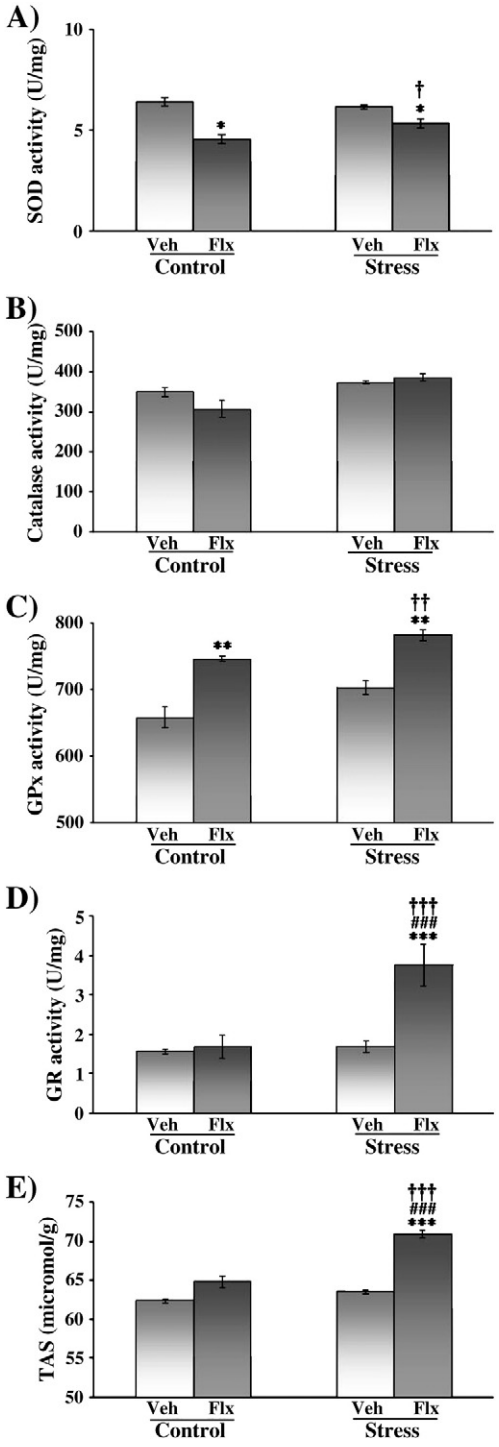

Fig. 1. Activity of antioxidant enzymes and total antioxidant status in the liver of control or chronicly stressed animals treated with fluoxetine. A) Superoxide dismutase (SOD) activity B) catalase activity C) glutathione peroxidase (GPx) activity, D) glutathine reductase (GR) activity and E) total antioxidant status (TAS). Animals in treatment groups received water (Veh) or fluoxetine (Flx) for 21 consecutive days, either alone (Control) or following 21 days social isolation (Stress). Data are presented as mean ± S.E.M. from 3 independent measurements of samples obtained from 2 separate groups of eight animals. *,**,***: $P<0.05$, $P<0.01$, $P<0.001$ compared with control, †,††,†††: $P<0.05$, $P<0.01$, $P<0.001$ compared with stressed group, ###: $P<0.001$ compared with fluoxetine treated group.

3.2. Protein expression of Bax and Bcl-2 in the liver

The balance of the expression of anti- and pro-apoptotic members of the Bcl-2 gene family dictates the susceptibility of the cells to a variety of apoptotic stimuli (Almeida et al., 2000). In order to learn whether stress or fluoxetine treatment can lead to cell death, the expression of proapoptotic Bax and antiapoptotic Bcl-2 protein in whole cell extracts was measured. For Bax protein expression, two-way ANOVA revealed a significant interaction between chronic stress and fluoxetine treatment [$F(1,36)=19.53$, $P<0.001$]. As shown in Fig. 2, we observed an increase of Bax protein expression upon fluoxetine treatment ($^{*}P<0.05$) or stress ($^{**}P<0.01$) with respect to untreated unstressed controls, as well as a

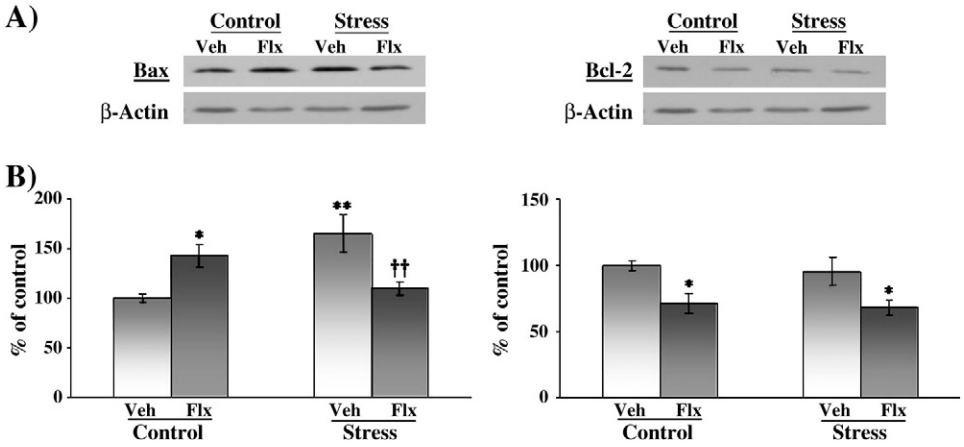

Fig. 2. Protein expression of Bax and Bcl-2 in the liver of control or chronicly stressed animals treated with fluoxetine. A) Representative Western blots and B) relative quantification by Image J analysis PC software of Bax and Bcl-2. The abbreviations are described in the caption of Fig. 1. Data are presented as mean ± S.E.M. from 3 independent measurements of samples obtained from 2 separate groups of eight animals. *,**: P<0.05, P<0.01 compared with control, ††: P<0.01 compared with stressed group.

decrease of Bax in chronically stressed group treated with fluoxetine with respect to the untreated stressed group (††P<0.01). Regarding Bcl-2, two-way ANOVA revealed a significant main effect only of fluoxetine treatment [$F(1,44) = 14.59$, $P<0.001$], and its protein expression was decreased in both groups treated with fluoxetine in comparison to the respective control groups (*P<0.05). In addition, we have calculated the relative ratios of Bax to Bcl-2 (Fig. 3). The ratio values above one indicated the dominance of Bax, while values below one suggested the prevalence of Bcl-2 protein. Two-way ANOVA revealed a significant main effect of the fluoxetine treatment [$F(1,34) = 4.91$, $P<0.05$], and the interaction between chronic stress and fluoxetine treatment [$F(1,34) = 15.47$, $P<0.001$]. The calculated ratios showed an increased Bax/Bcl-2 ratio in the group treated with fluoxetine (***P<0.001) or chronic stress (**P<0.01) and in stressed animals treated with fluoxetine (*P<0.05).

3.3. DNA fragmentation

We have analysed total genomic DNA isolated from the liver of the four experimental groups of Wistar rats, looking for the fragments seen after DNA cleavage associated with apoptosis (Filipkowski et al., 1994). Two-way ANOVA revealed a significant main effect of fluoxetine treatment [$F(1,12) = 12.44$, $P<0.001$]. As shown in Fig. 4, the intergroup comparison showed that only the increase of DNA fragmentation in the chronically stressed group treated with fluoxetine was significant with respect to the untreated stressed group (**P<0.01), while in the unstressed group treated with fluoxetine this increase was marginally significant (P = 0.069).

4. Discussion

In the experimental animals, chronic stress paradigms are considered as a model of depression (Willner, 1997), and in that context are used for examining the effects of different drug treatments. Since oxidative stress is an established outcome of chronic stress (Madrigal et al., 2001; Kaushik and Kaur 2003; Zafir and Banu, 2009), antioxidant defense is a very important aspect in the studies of stress related disorders and their treatment. Addressing this problem, our recent study (Djordjevic et al., 2010) reported a compromised functional state of liver antioxidant defense under chronic stress, whereas the present research was designed to investigate the effect of fluoxetine on antioxidant status and apoptotic signalling in the liver, a central organ for the activation and detoxification of many xenobiotics and reactive oxygen species. We have also investigated whether chronic fluoxetine treatment, in which 21 days is considered to be the time course of therapeutic action of the antidepressant, has the same effects in the liver of chronically stressed and unstressed animals with respect to these parameters.

We observed that the effects of fluoxetine on the antioxidant enzymes, SOD (downregulation) and GPx (upregulation), were the same in both unstressed and stressed animals, while those on GR activity (marked upregulation) and TAS (marked increase) were observed only in stressed animals (Fig. 1). Zafir and Banu (2007) have previously reported that treatment with fluoxetine can prevent psychological stress-induced oxidative damage by elevation of SOD activity in the brain, as well as, in the liver, but their result was not confirmed in our study. Specifically, we found that SOD activity was slightly downregulated after fluoxetine treatment, in both unstressed and stressed animals, which corresponds better to the results of human studies (Galecki et al., 2009; Khanzode et al., 2003). Giving the predominant role of SODs in scavenging superoxide anion to the hydrogen-peroxide, both quantitative depletion of the SODs or inhibition of their scavenging mechanisms, could lead to

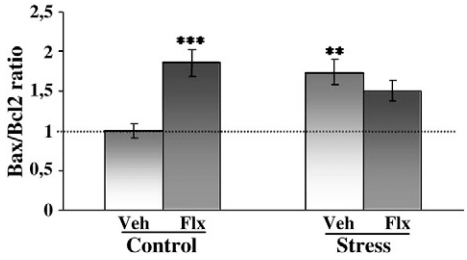

Fig. 3. Bax to Bcl-2 protein ratio in the liver of control or chronicly stressed animals treated with fluoxetine. The abbreviations are described in the caption of Fig. 1. Data are presented as mean ± S.E.M. from 3 independent measurements of samples obtained from 2 separate groups of eight animals. **,***: P<0.01, P<0.001 compared with control.

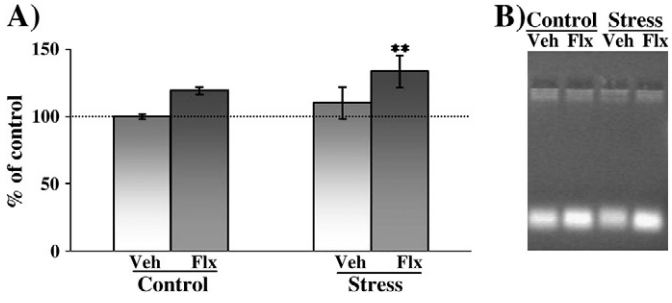

Fig. 4. DNA fragmentation in the liver of control or chronicly stressed animals treated with fluoxetine. A) Relative quantification of fragmented DNA performed by Image J analysis PC software and B) fragmented DNA separated from unfragmented chromosomal DNA analysed on 1.6% agarose gel. The abbreviations are described in the caption of Fig. 1. Data are presented as mean ± S.E.M. from 3 independent measurements of samples obtained from 2 separate groups of eight animals. **: P<0.01, compared with control.

generation of reactive oxygen species (in this case superoxide anion). Superoxide anion, if not eliminated by dismutation, can activate a number of signaling pathways that compromise cell redox balance and lead to oxidative stress. Even though the SOD, as main scavenger of the superoxide anion to the hydrogen-peroxide was downregulated (Fig. 1), we found that the activity of the hydrogen-peroxide metabolizing enzyme catalase remained unaffected, while enzymatic activity of GPx was induced under fluoxetine, regardless of whether it was applied to unstressed or stressed animals. The increase in GPx activity is known to emerge as a compensatory response to enhanced lipid peroxidation, since GPx scavenges primarily lipid peroxides. Therefore, the explanation for its elevation upon fluoxetine treatment could be interpreted in view of the research of Souza et al. (1994) who showed that fluoxetine directly interrupts the structure of the mitochondrial membrane in the hepatocytes. Moreover, such effect of fluoxetine in our study might also be potentiated by the decrease of SOD activity, since this enzyme is responsible for prevention of lipid peroxidation in the liver (Zimmermann et al., 1973) (Fig. 1A).

Interestingly, GR activity was evoked by the fluoxetine only in stressed animals, and not in the controls (Fig. 1D), suggesting that fluoxetine interferes with the stress-induced pathways of oxidative damage. It is possible that increased GR activity reflects higher requirements for reduced glutathione (GSH), which are needed for the protection against reactive oxygen compounds; it is well known that the GSH system is one of the main defense mechanisms involved when the liver is subjected to toxic aggression (Grattagliano et al., 2002), and that one of the fluoxetine metabolites depletes intracellular glutathione pull in rat liver (Thompson et al., 2000).

In addition, a significant increase in TAS was also detected only in stressed animals after fluoxetine treatment (Fig. 1E), which is in accordance with the results gathered from some human studies (Galecki et al., 2009). Since TAS determines the cooperative potential of mainly nonenzymatic antioxidants (such as uric acid, ascorbic acid, sulphydryl groups, tocopherol) to scavenge and neutralize reactive oxygen species, its increase may be part of a mechanism which leads to attenuation of reactive oxygen species-related reactions. Due to the fact that TAS increase was observed only in stressed animals treated with fluoxetine and not in unstressed ones, it could be correlated with the reduction of chronic stress effects. Our finding may also be interpreted in view of reports showing that SSRIs are effective within the context of clinical condition, such as stress history, and after chronic application (Lucassen et al., 2004). The antioxidant effect of fluoxetine (Kirkova et al., 2010; Zafir and Banu, 2007; Bilici et al., 2001) might also be related to the fact that this drug increases the level of serotonin, which has been reported to display antioxidant effects (Huether and Schuff-Werner, 1996). Nevertheless, our data of TAS increase (Fig. 1E) may also serve as a hallmark of increased oxidative insult in the liver, which was present under described experimental conditions. Regarding GR and TAS activity (Fig. 1D and E), it seems that fluoxetine does not exert antioxidant effects in the absence of oxidative stress conditions; rather, it directly interferes with stress-induced pathways of oxidative damage. Therefore, limited alterations in the activity of antioxidant enzymes and marked increases of TAS and GR activity in the liver of stressed animals treated with fluoxetine are indicative of enhanced oxidative insult, where nonenzymatic antioxidative defense plays a key role.

Although the increase in antioxidant defense is interpreted as antioxidant effect of the treatment it is also the hallmark of enhanced oxidative insult present under these conditions, and oxidative stress is the well known initiator of the apoptotic signaling. It is known that oxidative stress can activate mitochondrial pathway of apoptosis through up-regulation of Bax and down-regulation of Bcl-2 (Herrera et al., 2001), so it was of interest to analyze these proteins in fluoxetine treated unstressed or chronically stressed animals. Our results showed significant decrease of antiapoptotic Bcl-2 protein upon fluoxetine treatment (Fig. 2), which is a known molecular event in the initiation of apoptosis (Gross et al., 1999). Namely, the main role of Bcl-2 is in prevention of apoptosis by blocking cytochrome c release from mitochondria (Yang et al., 1997). Although fluoxetine differently influenced Bax protein level in unstressed and stressed animals (Fig. 2), the calculated ratios between Bax and Bcl-2 (Fig. 3) confirmed predominance of Bax in all groups. The prevalence of proapoptotic Bax over antiapoptotic Bcl-2 may be interpreted as activation of apoptotic response (Murphy et al., 2000). Increased DNA fragmentation, which is often seen in the cells undergoing apoptosis, was evident in our experiments only after fluoxetine treatment, and it was more pronounced in stressed animals (Fig. 4). Our findings regarding DNA fragmentation upon fluoxetine treatment correspond better to a decrease of Bcl-2, than to an increase in the Bax-to-Bcl-2 ratio (Figs. 2 and 3). They are also in accordance with findings of other authors that observed reduced expression of Bcl-2 protein and induced apoptosis under fluoxetine (Zhai et al., 2009). Here it is interesting to point out that, based on the documented apoptotic potential of fluoxetine, this drug was also considered as a promising therapeutic for highly aggressive Burkitt lymphoma (Serafeim et al., 2003).

In the light of the findings presented herein, most pronounced DNA fragmentation was seen upon fluoxetine treatment of stressed animals. We believe that stress related oxidative damage, judged by the increase in TAS and GR activity, could have primed fluoxetine effects on hepatic apoptosis. In a clinical setting, it is of special importance to know all the factors, chronic psychosocial stress being one, that can modulate the activities of liver enzymes and thus affect the rate of fluoxetine metabolism and the final outcome of fluoxetine treatment. The results of the present study shed more light on pharmacological modulation of antidepressant fluoxetine by oxidative stress.

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