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Lack of changes in beta-endorphin plasma levels after repeated treatment with fluoxetine: possible implications for the treatment of alcoholism – a pilot study

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Clinical and animal studies indicate that selective serotonin-reuptake inhibitors (SSRIs) may help to reduce alcohol intake but investigations led to conflicting results. A few studies indicated that serotonin (5-HT) may modulate the brain beta-endorphin level, which plays an important role in the development of alcohol craving. Our study examined the influence of fluoxetine on the endogenous opioid system. We investigated plasma levels of beta-endorphin in rats with either high alcohol preference (Warsaw High-Preferring; WHP) or low alcohol preference (Warsaw Low-Preferring; WLP) after repeated treatment with fluoxetine (5 mg/kg i.p. for 21 days). We examined the rats 24 hours after fluoxetine treatment in order to determine whether chronic fluoxetine produces a long-term change in the beta-endorphin levels. The animals received either a single dose of ethanol (2 g/kg) or an identical single dose of saline one hour before blood collection. While a few studies observed an increase in the level of beta-endorphin after a single fluoxetine injection, we did not observe any increase in beta-endorphin plasma levels after repeated fluoxetine treatment. We also did not observe any changes in beta-endorphin levels of rats treated with fluoxetine and injected with ethanol. A lack of increase of beta-endorphin levels may explain why fluoxetine has a limited value in the prevention of craving for alcohol.

1. Introduction

Many studies have suggested that dysfunction of the central serotonergic system predisposes individuals to excessive drinking (Berggren et al. 2002; Heinz et al. 2001, 1998; Balldin et al. 1994). It has been suggested that serotonin has a role in the craving often seen in alcoholics (Johnson et al. 2002; Ciccocioppo 1999). It is also known that human alcoholics and alcohol-preferring animals have lower levels of serotonin (5-HT) than nonalcoholics (Javors et al. 2005; Lovinger 1997; Zhou et al. 1994). It was therefore postulated that reduced central 5-HT levels are involved in mediating the alcohol preference in animals (LeMarquand et al. 1994). Measurements of the density of 5-HT receptors and of the concentrations of 5-hydroxyindoleacetic acid (5-HIAA), which is a major metabolite of 5-HT, in various brain regions of animals have shown that both are lower in alcohol-preferring rats than in non-preferring ones (Strother et al. 2005; Ciccocioppo et al. 1998, 1997; Devoto et al. 1998; McBride et al. 1993). Some human studies also support a relationship between alcohol consumption and reduction in 5-HT transmission (Heinz et al. 1998; Borg et al. 1985). Clinical and experimental observations indicate that medications that affect the serotonergic system provide benefit in the treatment of alcoholism and that selective serotonin-reuptake inhibitors (SSRIs) may reduce alcohol intake and craving in some groups of alcoholics (Naranjo et al. 1994). However, SSRIs seem to have only a modest effect in reducing alcohol consumption.

Serotonin activity and receptor-reuptake blockade are thought to be closely related to the analgesic effect that seems to be due to an increase in endogenous opioid activity in the brain (Sacerdote et al. 1987). Endogenous opioid peptides, especially beta-endorphins, are functionally connected to a dopamine reward system, which has the most important role in alcohol addiction (Zalewska-Kaszubska and Czarnecka 2005). Some studies suggest that endorphins may be implicated in craving for addictive drugs (van Ree et al. 1994, 1996; Kiefer and Wiedemann 2004). The reward system is also highly innervated by serotonin afferents from the raphe nuclei to the ventral tegmental area and nucleus accumbens. Serotonin regulates and modulates dopaminergic function in the dopamine forebrain projection system, and it may indirectly modulate dopaminergic brain substrates relating to drug-induced reward and relapse (Walsch and Cunningham 1997). Some results have indicated that 5-HT may modulate the levels of brain beta-endorphin (Petraglia et al. 1984). Sapun et al. (1981) investigated the serotonergic control of the pituitary beta-endorphin secretion in the rat and found that fluoxetine and quipazine (a serotonin re-

ceptor agonist) treatment increased plasma beta-endorphin levels. This finding was confirmed by other studies (Carr et al. 1991; Bruni et al. 1982). Petraglia et al. (1984) found that fluoxetine induced a significant dose-dependent increase in beta-endorphin and beta-lipotropin levels in humans.

We have previously found that acamprosate and naltrexone, which are the most effective alcoholism treatments, increase the levels of beta-endorphin (Zalewska-Kaszubska et al. 2005, 2006). We found a different release pattern of this peptide in rats selectively bred for alcohol preference (Warsaw High-Preferring; WHP) compared with those bred for non-preference of alcohol (Warsaw Low-Preferring; WLP). The neurochemical studies proved that, in the WHP rats, concentrations of serotonin and dopamine, as well as their metabolites, were lower than in the WLP rats (Dyr and Kostowski 2004).

The current experiments were designed to investigate changes in the beta-endorphin plasma levels in long-term fluoxetine-treated rats, specifically WHP and WLP rat lines, as well as to investigate the effect of a single dose of alcohol in these rats.

2. Investigations, and results

Both WHP and WLP rats that had been treated for 21 days with saline showed statistically significant increase of beta-endorphin plasma levels after an injection of alcohol (2 g/kg body weight) (Fig.). A single application of ethanol caused the beta-endorphin plasma level to increase from 236 ± 20 pg/ml to 568 ± 57 pg/ml (140%) and from 417 ± 53 pg/ml to 614 ± 58 pg/ml (47%) in WHP and WLP rats, respectively. In a three-way analysis of variance, which included strain × fluoxetine treatment × ethanol treatment, three two-way interaction effects were observed, but only the strain × alcohol treatment variance showed a statistically significant interaction effect $F(1,40) = 4.83$, $p < 0.05$. Fluoxetine administered intraperitoneally for 21 days in the dose of 5 mg/kg body weight caused neither in WHP rats (230 ± 45 pg/ml) nor in WLP rats (416 ± 24 pg/ml) any statistically significant increase of beta-endorphin plasma level. Whether subject to a 21-day treatment with fluoxetine or saline, the effect of a single application of ethanol on the level of beta-endorphin was quite similar both in WLP rats (637 ± 69 pg/ml) and WHP rats (621 ± 42 pg/ml). Post-hoc LSD test has shown significant differences between WHP and WLP rats (Fig.). The extraction recovery for beta-endorphin was 75 ± 7%.

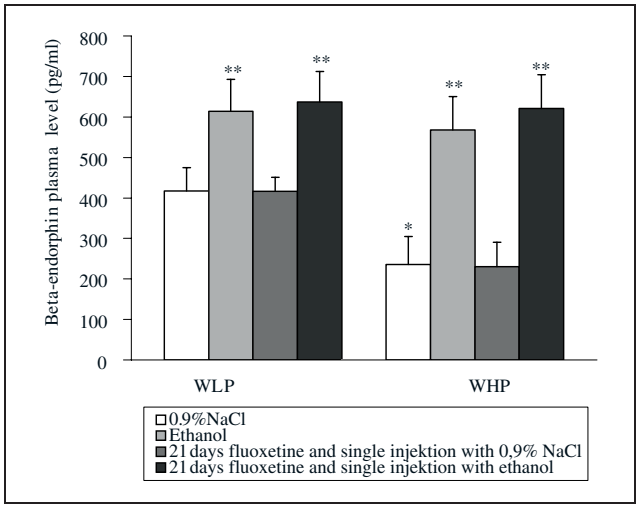

Fig.: Effect of single injection of ethanol (2 g/kg) on β-endorphin plasma level in WLP and WHP after 21 days of administration of fluoxetine. Values are expressed as the mean ± SEM in each group of 6 rats.
* $p < 0.05$ in comparison to WLP group injected with 0.9% NaCl
** $-p < 0.05$ in comparison to respective WLP or WHP group injected with 0.9% NaCl

3. Discussion

In this study we investigated the effect of a 21-day fluoxetine treatment on the beta-endorphin plasma levels in adult WHP and WLP female rats. Basal beta-endorphin levels were significantly lower in WHP rats than in WLP rats. A single injection of ethanol resulted in similar plasma beta-endorphin levels in both lines of rats, however the percentage of increase of plasma beta-endorphin levels was different for WHP and WLP lines in comparison with each line's respective control group. For the WHP line the increase was 141% while for the WLP rats the increase was of 47%. Studies of human subjects also showed differences in the increase of beta-endorphin in individuals with a family history of alcoholism in comparison to individuals without such history (Gianoulakis et al. 1989).

A few studies have reported an increase in beta-endorphin levels through the stimulation of the serotonergic system. Sapun et al. (1981) found that in rats, quipazine (a serotonin receptor agonist), increased the plasma beta-endorphin levels for approximately 15–45 min after its administration. Zangen et al. (1999) reported that an increase in extracellular beta-endorphin level in response to a single dose of fluoxetine lasted only 1–1.5 h in the arcuate nucleus and nucleus accumbens of rats. In the present study we measured the level of this peptide 24 h after the last of repetitive fluoxetine administrations, the same way we measured the level of beta-endorphin in our earlier studies with acamprosate and naltrexone (Zalewska-Kaszubska et al. 2005, 2006). Differences in beta-endorphin plasma levels after the 21st day fluoxetine treatment occurred neither in the WHP rats nor in the WLP rats. Our study confirms the results of an experimental work, which reported that in alcohol-preferring rats, administration of fluoxetine for 7 consecutive days led to a significant reduction in ethanol self-administration, but when fluoxetine treatment was terminated, ethanol self-administration quickly returned to the pre-treatment levels (Murphy et al. 1988). Our study suggests that no adaptive changes in beta-endorphin levels occur in animals from which fluoxetine had been withdrawn, despite the fact that some norfluoxetine was probably still present in the plasma $t_{1/2} \sim 15$ hours). Although Murphy et al. (1988) did not assess the effect of beta-endorphin our study seems to confirm their findings. Our results also confirm clinical observations of Gorelick and Parades (1992), in which a reduction of alcohol consumption under fluoxetine influence was reported to be short-lived. We infer that the beta-endorphin level increase may be observed only if a large concentration of fluoxetine is still present in the rats' blood. The present pilot study was based on a limited number of fluoxetine doses and more doses need to be examined in subsequent studies.

A few studies, which did not measure the level of beta-endorphin, reported negative results of fluoxetine in alcohol therapy. Patkar et al. (2003) reported results of preclinical studies that do not support the involvement of a serotonergic mechanism in alcohol withdrawal and craving. In addition, Kranzler et al. (1995) reported the results of a clinical placebo-controlled study that did not support the theory that fluoxetine treatment reduces relapse frequency

compared with placebo. However they found that fluoxetine may reduce depressive symptoms in alcoholics with major depression.

On the basis of the literature and our earlier studies with acamprosate and naltrexone, we postulate that the levels of beta endorphin is a very important factor in prevention of craving, and prevention of relapse in alcohol-dependent subjects. It seems that the lack of a long lasting effect on the beta-endorphin levels may limit fluoxetine application in alcohol therapy. Nonetheless, fluoxetine may be useful in treatment of alcoholics with depressive symptoms.

4. Experimental

4.1. Animals

The experiments were carried out on female adult rats from the F_{34-36} generation of the WHP and WLP rat lines, which weighed 240–320 g. All animals were between 4 and 6 months of age. Female rodents overall consume more ethanol than males (Jones and Whitfield 1995). While fluctuation in alcohol intake over the estrus cycle have been reported by Roberts et al. (1998) numerous other studies showed that ethanol administration had no effect on estrus cycle (Roberts et al. 1998; van Haaren and Anderson 1994). Moreover, female rats housed together tend to largely synchronize cycles within the time of the experiment (Wiren et al. 2006). The animals were kept under standard laboratory conditions.

4.2. Procedures

Forty eight rats were distributed into eight groups with six animals in each group. Four groups consisted of WHP rats (24 animals) and four groups of WLP rats (24 animals). Two of the WHP groups (12 animals) and two of WLP groups (12 animals), received fluoxetine for 21 days (5 mg/kg; 0.2 ml/100 g body weight, daily) intraperitoneally. Fluoxetine in a dose of 5 mg/kg has been reported as being effective in reducing ethanol intake and in ethanol withdrawal syndrome in rats (Martijena et al. 2005; Uzbay et al. 2004). The mean elimination half-live ($t_{1/2}$) of fluoxetine and of its active metabolite norfluoxetine in rats is about 5 and 15 h, respectively (Caccia et al. 1990). Before a blood collection, six rats from the WHP group and six rats from WLP group were injected with ethanol whereas the remaining six WLP and six WHP rats were injected with the same volume of saline. The other 24 rats (12 WHP and 12 WLP rats) had been treated for 21 days with the same volume saline only and before a blood sample was collected they received an injection of ethanol or saline in the same way as the fluoxetine treated rats. A blood sample was collected 1 h after ethanol administration as the rats' blood alcohol concentration would be the highest one hour after i.p. ethanol treatment (Marinelli et al. 2003) and during the time period of 60 to 90 min after ethanol administration a maximal release of beta-endorphin can be observed (Olive et al. 2001).

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Research Committee.

4.3. Materials

Sep-pak C 18 cartridges were obtained from Waters M.A., USA cat. No. WAT 020515; acetone (HPLC grade) and trifluoroacetic acid (HPLC grade) were from Baker. Aprotinin (Trascolan®) was purchased in Jelfa, Poland; fluoxetine hydrochloride was from Synteza, Poland. Ether was purchased in POCh, Poland. The plasma beta-endorphin radioimmunoassay kit was obtained from Phoenix Pharmaceuticals, Inc. USA.

4.4. Blood sample procedure

The rats were anaesthetized with ether 24 h after the last administration of fluoxetine or saline and the blood samples were collected by heart puncture. The rats were injected i.p. with ethanol (20% w/v; 2 g/kg/10 ml) or the same volume of saline 1 h before blood sample collection.

Blood was sampled in tubes containing EDTA (1.6 mg/ml) and gently rocked several times to prevent coagulation. Then, the samples were transferred to centrifuge tubes containing aprotinin (500 KIU/ml) and gently rocked several times to inhibit proteinases activity. The samples were cooled in an ice-bath immediately afterwards. The plasma was separated by centrifugation at $1\,600 \times g$ for 15 min at 4 °C. The plasma was frozen and stored at −20 °C until assessment.

4.5. Solid phase extraction of peptides from plasma

Plasma beta-endorphin levels were determined after extraction by the acid-acetone method. The procedure for beta-endorphin extraction was based on the use of Sep-pak C 18 cartridges in according to the method by Angwin and Barchas (1982) and modified by Zalewska-Kaszubska and Obzejta (2004).

Before loading on Sep-Pak C-18 cartridges, plasma was acidified in the same volume of 1% trifluoroacetic acid (TFA) and centrifuged at $10000 \times g$ for 20 min at 4 °C. C-18 Sep-columns were activated by passing 2 ml of acetone through them and subsequently equilibrated twice with 2 ml of 1% TFA in distilled water. The supernatant of acidified plasma solution was loaded onto the columns. The columns were washed twice with 2 ml of 1% TFA. Beta-endorphins were eluted with 1.5 ml of 1% TFA/acetone (25 : 75) and dried under vacuum.

Plasma levels of beta-endorphin were estimated with radioimmunoassay, using kit supplied by Phoenix Pharmaceuticals, Inc. USA.

4.6. Statistical analysis

All data were expressed as mean ± SEM. Statistical analysis was performed using three way analysis of variance followed by post-hoc LSD (Least Significant Differences). Normal distribution of data was tested using the Kolmogoroff-Smirnov test with Lillieforse correction. Differences were considered significant at $p < 0.05$.

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