Turk J Vet Anim Sci
29 (2005) 857-863

Research Article

The Effects of Fenfluramine on Blood and Tissue Serotonin (5-Hydroxytryptamine) Levels in Rats*

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Received: 19.04.2004

Abstract: The aim of this study was to investigate the effect of fenfluramine (FEN) administered at single and repeated doses on brain, stomach and plasma serotonin (5-Hydroxytryptamine, 5-HT) levels in rats. FEN was injected intraperitoneally at single doses (1, 3 and 10 μmol kg^{-1}) and given orally at repeated doses (1.25, 5 and 10 mg kg^{-1}) for 21 days. The tissues and plasma samples were collected 20, 40, 80 and 160 minutes after the single dose administrations and 4 h after the last dose administration in repeated experiments. In addition, the rats treated with the dose of 1.25 mg kg^{-1} were maintained until 2 and 8 weeks and the tissues were taken at these times. The 5-HT levels in the tissue and plasma were measured by using high performance liquid chromatography (HPLC).

The levels of brain 5-HT were decreased at the single FEN doses when compared with the control group. At the repeated experiments, although no effect was observed at the dose of 1.25 mg kg^{-1}; decreases were determined in brain 5-HT levels at the high doses. The levels of 5-HT in the stomach were found to increase in both single and repeated administrations. Decreases in levels of plasma 5-HT were determined in all experimental groups. In conclusion, while fenfluramine was determined to decrease brain and plasma 5-HT levels generally, it increased the levels of stomach 5-HT.

Key Words: Fenfluramine, rat, brain, stomach, plasma, serotonin.

Ratlarda Fenfluraminin Kan ve Doku Serotonin (5-Hidroksitriptamin) Düzeyleri Üzerine Etkileri

Özet: Bu çalışmanın amacı, ratlarda tek ve tekrarlanan dozlarda uygulanan fenfluramin (FEN)'in beyin, mide ve plazma serotonin (5-Hidroksitriptamin, 5-HT) düzeyleri üzerine olan etkilerinin belirlenmesidir. FEN hayvanlara periton içi yolla tek doz (1, 3, 10 μmol/kg) ve ağızdan 21 gün süreyle tekrarlanan dozlar (1,25, 5 ve 10 mg/kg) şeklinde uygulandı. Tek doz uygulamalarını takiben 20, 40, 80 ve 160. dakikalarda; uzun süreli uygulamalarda ise 21 günlük uygulamanın bitmesinden 4 saat sonra dokular alındı. Ayrıca, 1,25 mg/kg dozunda uygulama yapılan gruptaki hayvanlar ilaç uygulaması tamamlandıktan sonraki 2 ve 8.haftalara kadar muhafaza edildi ve dokuları alındı. Doku ve plazma 5-HT düzeyleri yüksek basınçlı sıvı kromatografi (HPLC) cihazında belirlendi.

Tek doz fenfluramin uygulaması yapılan gruplarda beyin 5-HT düzeylerinin kontrol grubuna göre azaldığı, tekrarlanan uygulamalarda ise FEN'in 1,25 mg/kg dozunda beyin 5-HT düzeyleri üzerine pek etkili olmadığı, buna karşılık yüksek dozlarda azalttığı görüldü. Mide 5-HT düzeylerinin hem tek hem de tekrarlanan uygulamalarda arttığı tespit edildi. FEN'in plazma 5-HT düzeylerinde ise belirgin bir şekilde azalmalara neden olduğu görüldü. Sonuç olarak FEN'in beyin ve plazma 5-HT düzeylerinde azalmaya; mide de ise artışlara neden olduğu belirlendi.

Anahtar Sözcükler: Fenfluramin, rat, beyin, mide, plazma, serotonin.

Introduction

5-HT is an indolic compound that is synthesized from the essential amino acid tryptophan. 5-HT synthesis rate is dependent on the levels of tryptophan in the diet. Important depots of 5-HT in mammals are enterochromaffin cells in the gastrointestinal mucosa, serotonergic neurons of the brain, pineal gland and platelets. 5-HT can be released from enterochromaffin

* This study was supported by the Fırat University Investigation Foundation (FÜNAF 349).

cells by acetyl-choline and noradrenergic nerve stimulation, increased intraluminal pressure and a decline of intestinal pH (1-6). 5-HT is involved in a variety of physiological processes, including smooth muscle contraction, blood pressure regulation and both peripheral and central nervous system neurotransmission. In the central nervous system, it acts as a neurotransmitter-neuromodulator that is implicated in sleep pattern regulation, appetite control, sexual activity and aggression. In the periphery, 5-HT acts as a vasoconstrictor and proaggregator when released from aggregating platelets. It also acts as a neurotransmitter in the enteric plexus of the gut and as an autocrine hormone when released from enterochromaffin cells from the gut, pancreas and elsewhere (7-10).

Fenfluramine (FEN), [N-ethyl-α-methyl-m-(trifluoromethyl)phenethylamine] is an appetite suppressant with proven efficacy. Two forms of the drug that exhibit similar pharmacology have been prescribed: the d, l-racemic mixture (FEN) and the more potent d-isomer (d-FEN). Despite structural similarities to amphetamine, FEN is not a locomotor stimulant and is seldom abused. FEN stimulates 5-HT release and inhibits 5-HT reuptake in brain tissue (11-15). In vivo microdialysis studies in rat brain demonstrate that systemic or local administration of FEN elevates extracellular levels of 5-HT. This effect of FEN is blocked by 5-HT reuptake inhibitors like fluoxetine. Although FEN influences both 5-HT release and reuptake, the 5 HT releasing capability of the drug seems to predominate in vivo (16).

The clinical use of FEN relates to its potential adverse effects on 5-HT neurons. It is shown that administration of FEN at sufficient doses can cause long-lasting depletion of 5-HT and degeneration of 5-HT nerve terminals throughout the forebrain in laboratory animals. Depletions of 5-HT are associated with a loss of 5-HT immunoreactive nerve fibers and a reduction in 5-HT reuptake sites. 5-HT transporter proteins play an important role in the release of 5-HT. Like other amphetamines, FEN release endogenous 5-HT by a carrier-mediated mechanism involving 5-HT transporter proteins in cell membranes (7,11,12,17-21).

The aim of this study was to evaluate the short- and long-term effects of FEN at single and repeated doses on brain, stomach and plasma 5-HT levels in rats.

Materials and Methods

Animals and Drug Administrations:

Wistar rats weighing 180-230 g were adapted to laboratory conditions before use and were maintained in a room with controlled temperature (20 ± 2 °C), relative humidity (60%) and 12 h light:12 h dark cycle. Rats were housed in plastic cages with free access to drinking water and basal diet. The rats were divided into two main groups as single and repeated FEN hydrochloride (Sigma F-8507) administered groups.

Single dose FEN administrations:

Group 1 (Control): (n = 12): Rats received intraperitoneal (ip) injection of 0.5 ml of distilled water.

Group 2 (n = 24): This group was injected with FEN 1 μmol kg^{-1} ip in 0.5 ml of distilled water.

Group 3 (n = 24): This group was injected with FEN 3 μmol kg^{-1} ip in 0.5 ml of distilled water.

Group 4 (n = 24): This group was injected with FEN 10 μmol kg^{-1} ip in 0.5 ml of distilled water.

Repeated FEN administrations:

Group 5 (Control) (n = 12): Rats received oral administration of isotonic saline for 21 days.

Group 6 (n = 18): This group was administered FEN 1.25 mg kg^{-1} orally for 21 days.

Group 7 (n = 18): This group was administered FEN 5 mg kg^{-1} orally for 21 days.

Group 8 (n = 18): This group was administered FEN 10 mg kg^{-1} orally for 21 days.

Sample Collection

The animals in groups of the single dose FEN administrations (Groups 1-4) were decapitated 20, 40, 80 and 160 minutes after the applications. On the other hand, the animals in the repeated experiments (Group 5-8) were decapitated 4 h after the last applications. In addition, the rats treated with the dose of 1.25 mg kg^{-1}, (Group 6) were maintained until 2 and 8 weeks by only placebo and the rats were decapitated at these times. The effects of FEN on body weight in Groups 5, 6, 7 and 8 were assessed by a laboratory balance before and after the experiments. The tissues and plasma samples were collected in all groups after the decapitations. Whole brain and stomach tissues were removed immediately and stored at -30 °C until the assay. Blood samples were

collected into tubes (5-7 ml) containing 2% sodium oxalate and the samples were centrifuged at 3000 rpm to separate their plasma and stored at -30 °C until the assay.

Determination of 5-HT Levels

5-HT creatinin sulfate (H-7752) and other chemicals and reagents were purchased from Sigma (St. Louis, MO, USA). The homogenization of tissues and plasma was carried out in a Teflon-glass homogenizer with a buffer containing 0.02% ascorbic acid in 0.1 M perchloric acid and centrifuged at 15,000 rpm for 15 min. The supernatants were pooled. The concentrations of 5-HT were quantified in the supernatant using HPLC (TSP, P4000 Quat gradient pomp, FL 3000 detector, AS 3000 autosampler) according to the published methods (22). After filtration through a 0.45 µ filter, 20 µl aliquots of clear supernatants were injected into the HPLC with a C 8 reversed phase column (Waters Assoc., stainless steel, 25 cm, 3.9 x 150 mm ID, consisting of 5 µ particle size octadecylsilica). Mobile phase consisting of 0.01 M acetate buffer (pH 4.3) in 30% methanol was circulated at a flow rate of 0.5 ml/min, column temperature 24 ± 1.0 °C. Excitation and emission wavelengths were set at 280 and 350 nm, respectively. The levels of 5-HT in the tissues were calculated using a computer program (OS/2 Systems) on HPLC.

Statistical Analysis

All results were expressed as mean ± SEM. Comparisons of groups were evaluated by one-way ANOVA followed by Duncan's test in SPSS for Windows. The results were considered statistically significant when $P < 0.05$.

Results

Tissue and plasma 5-HT levels in animals treated with single and repeated doses of FEN are shown in Tables 1-6. Treatment with a single dose of FEN caused significant decreases in brain 5-HT levels at 20 min when compared with the control group and these levels were observed to be at the minimal level at 160 min (Table 1). After the repeated administrations of FEN at 4 hours, no change was detected in brain 5-HT levels in Group 6 when compared with the control group, but slight decreases were observed in Groups 7 and 8. On the other hand, brain 5-HT levels at 2 and 8 weeks were decreased by FEN in Group 6 (Table 2).

When compared with the control group administration of single doses of FEN to rats caused decreases in stomach 5-HT levels in Group 2, but increases were observed in Groups 3 and 4 at 20 min. However, increases in stomach 5-HT levels were

Table 1. Brain 5-HT levels (ppb) in rats treated with single doses of FEN.

	Group1 (Control)	Group 2 (1µmol kg^{-1})	Group 3 (3 µmol kg^{-1})	Group 4 (10 µmol kg^{-1})
20th min	54.9 ± 3.2[a]	38.2 ± 2.2[b]	34.2 ± 3.0[c]	30.4 ± 3.4[c]
40th min	54.9 ± 2.9[a]	47.8 ± 4.2[a]	28.5 ± 2.4[b]	35.7 ± 2.5[b]
80th min	53.3 ± 1.8[a]	41.3 ± 2.8[b]	27.9 ± 3.3[c]	26.1 ± 1.7[c]
160th min	54.0 ± 2.5[a]	29.3 ± 2.6[b]	23.3 ± 3.6[b]	25.2 ± 3.4[b]

[a, b, c] : The differences between values marked with different letters on the same line are significant. $P < 0.05$ (Compared to control group).

Table 2. Brain 5-HT levels (ppb) in rats treated with repeated doses of FEN.

	Group 5 (Control)	Group 6 (1.25 mg kg^{-1})	Group 7 (5.0 mg kg^{-1})	Group 8 (10 mg kg^{-1})
4th hour	54.9 ± 3.5[a]	54.6 ± 3.4[a]	45.9 ± 5.1[b]	44.8 ± 2.4[b]
2nd week	53.2 ± 1.4[a]	34.1 ± 3.9[b]	-	-
8th week	55.9 ± 4.2[a]	29.8 ± 1.9[b]	-	-

[a, b, c] : The differences between values marked with different letters on the same line are significant. $P < 0.05$ (Compared to control group).

observed at 40, 80 and 160 min when compared with the control group in Groups 3 and 4 (Table 3). Repeated administrations of FEN caused increases in stomach 5-HT levels at 4 h. in all groups according to the control group. Similarly, significant increases were also detected in stomach 5-HT levels at 2 and 8 weeks in Group 6 (Table 4).

Significant decreases in plasma 5-HT levels were observed in both single and repeated administrations of FEN to rats in all times when compared with the control groups (Tables 5 and 6).

The changes in body weights of animals treated with the repeated doses of FEN are shown in Table 7. A significant increase in body weight (17.1%) was observed in the control group (Group 5) while significant decreases (21.7%, 9.3% and 29.9%) were noted in Groups 6, 7 and 8, respectively.

Discussion

The amphetamine derivatives d, l-FEN and d-FEN had been widely used as appetite suppressants until their removal from the market due to serious side-effects. High doses of FEN cause long term degeneration of 5-HT nerve terminals throughout the forebrain. These degenerations include depletion of tissue 5-HT, decreased 5-HT biosynthesis, and loss of 5-HT transporters (SERT) (12,13). Recently, it has been shown that FEN causes the

Table 3. Stomach 5-HT levels (ppb) in rats treated with single dose of FEN.

	Group 1 (Control)	Group 2 (1μmol kg^{-1})	Group 3 (3 μmol kg^{-1})	Group 4 (10 μmol kg^{-1})
20th min	489 ± 34[a]	446 ± 33[a]	533 ± 16[b]	656 ± 36[c]
40th min	467 ± 41[a]	651 ± 35[b]	749 ± 54[c]	444 ± 39[a]
80th min	480 ± 22[a]	687 ± 32[ab]	770 ± 45[c]	617 ± 43[b]
160th min	472 ± 44[a]	651 ± 27[b]	754 ± 22[c]	689 ± 19[ab]

[a, b, c] : The differences between values marked with different letters on the same line are significant. $P < 0.05$ (Compared to control group).

Table 4. Stomach 5-HT levels (ppb) in rats treated with repeated doses of FEN.

	Group 5 (Control)	Group 6 (1.25 mg kg^{-1})	Group 7 (5.0 mg kg^{-1})	Group 8 (10 mg kg^{-1})
4th hour	477 ± 54[a]	774 ± 28[b]	895 ± 34c	953 ± 63c
2nd week	526 ± 40[a]	696 ± 39[b]	-	-
8th week	467 ± 38[a]	726 ± 51[b]	-	-

[a, b, c] : The differences between values marked with different letters on the same line are significant. $P < 0.05$ (Compared to control group).

Table 5. Plasma 5-HT levels (ppb) in rats treated with single dose of FEN.

	Group 1 (Control)	Group 2 (1μmol kg^{-1})	Group 3 (3 μmol kg^{-1})	Group 4 (10 μmol kg^{-1})
20th min	546 ± 40[a]	235 ± 37[b]	260 ± 13[b]	311 ± 22[c]
40th min	502 ± 22[a]	294 ± 31[b]	463 ± 26[c]	354 ± 32[b]
80th min	523 ± 16[a]	280 ± 29[b]	434 ± 34[c]	463 ± 34[c]
160th min	549 ± 25[a]	281 ± 30[b]	251 ± 17[b]	203 ± 18[b]

[a, b, c] : The differences between values marked with different letters on the same line are significant. $P < 0.05$ (Compared to control group).

Table 6. Plasma 5-HT levels (ppb) in rats treated with repeated doses of FEN.

	Group 5 (Control)	Group 6 (1.25 mg kg^{-1})	Group 7 (5.0 mg kg^{-1})	Group 8 (10 mg kg^{-1})
4th hour	676 ± 34[a]	484 ± 14[b]	341 ± 41[c]	241 ± 29[c]
2nd week	567 ± 44[a]	542 ± 21[a]	-	-
8th week	641 ± 37[a]	332 ± 33[b]	-	-

[a, b, c] : The differences between values marked with different letters on the same line are significant. $P < 0.05$ (Compared to control group).

Table 7. Changes of body weight (%) in rats treated with repeated doses of FEN.

	Body Weight at the Beginning of Experiments (g)	Body Weight at the End of Experiments (g)	Changes in Body Weight (%)
Group 5 (Control)	156.6 ± 17[a]	183.3 ± 14[b]	+ 17.1
Group 6	190.0 ± 22[a]	148.8 ± 26[b]	- 21.7
Group 7	230.0 ± 31[a]	213.3 ± 19[a]	- 09.3
Group 8	223.1 ± 12[a]	156.6 ± 13[b]	- 29.9

[a, b] : The differences between values marked with different letters on the same line are significant. $P < 0.05$ (Compared to control group).

release of 5-HT from nerve terminals and inhibits 5-HT reuptake in in vitro investigations (14,17). However, in vivo microdialysis studies demonstrated that administration of FEN elevates extracellular levels of 5-HT in intact brain. Like other amphetamines, FEN releases endogenous 5-HT by a carrier-mediated mechanism involving SERT proteins in cell membranes (16,23-25). In our study, the levels of 5-HT in the brain have been shown to decrease when compared with the control group, especially at the single high doses (3 and 10 μmol kg^{-1}) of FEN in rats. These observations indicated that FEN may decrease 5-HT levels in the rat brain due to the inhibition of 5-HT reuptake and to the stimulation of 5-HT release from nerve cells.

Caccia et al. (19) reported that brain 5-HT levels decreased after 4 h in 3-week administration of FEN the same as acute applications dependent on doses. In addition, two weeks after 1.25 mg kg^{-1} of FEN was decreased to minimal levels but increases in brain 5-HT levels showed at 8 weeks. In this study, although no important changes were observed in brain 5-HT levels at repeated administration of 1.25 mg kg^{-1} FEN, significant decreases were determined at the doses of 5 and 10 mg kg^{-1} when compared with the control group. However, decreases in 2 and 8 weeks after 1.25 mg kg^{-1} in brain 5-HT levels were noted. The observations of the present study are in accordance with the findings of Caccia et al. (19) and indicated that repeated administration of FEN causes prolonged depletion of brain 5-HT.

The majority of 5-HT is found in enterochromaffin cells of the gastrointestinal tract in animals. In these structures, it accounts for approximately 80% of total body 5-HT content. Released 5-HT from these cells by stimulations can be detected in intestinal lumen and systemic circulation (1,15). Costa et al. (15) showed that 5-HT levels in the stomach increased slightly at high doses of FEN in rats. Our results also show that single doses of FEN increased stomach 5-HT levels irregularly, but repeated administrations of FEN elevated these levels dependent on doses. These results for the effects on stomach 5-HT levels of FEN are in accordance with the findings of the above researchers.

The majority of platelet 5-HT originates from enterochromaffin cells. Circulation plasma 5-HT is taken up by platelets mainly by an active transport mechanism. In the circulation, it is almost entirely confined to platelets and thereby rendered functionally inactive. Some investigators (2,26-28) showed that carcinoid tumors,

during long-term 5-HT rich-foods ingestion and long-term FEN administrations, caused an increase in circulating plasma. However, 5-HT is stored in platelets and circulating 5-HT levels are therefore decreased. Finally, a decrease in circulating 5-HT levels was determined. Our results for the circulating 5-HT levels showed that FEN may decrease circulating 5-HT levels in rats due to storage in platelets. The observation of the above researchers is in agreement with the results of the present study.

In rats, FEN is quite effective in reducing food consumption, possibly because of its ability to enhance serotoninergic transmission by potentiating the release of 5-HT and inhibiting its inactivation by reuptake. Investigators showed that long-term FEN administrations caused a dramatic reduction of body weight gain (10,12,17,20). The decreases in body weight observed in this study are in accordance with the reports of the above researchers and can be explained by the effects of FEN on brain 5-HT levels.

In conclusion, we showed that single and long-term administrations of FEN induced decreases in brain and plasma 5-HT levels but increased stomach levels. Therefore, significant decreases were observed in body weight in long-term administrations of FEN, especially at high doses.

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