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ARAŞTIRMA / RESEARCH

Vasoactive effects of fluoxetine in rat thoracic aorta smooth muscle

Fluoksetinin sıçan torasik aort düz kasındaki vazoaktif etkileri

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Abstract

Purpose: While most studies of fluoxetine have focused on its effects on the cardio/cerebrovascular systems, what is known about its vasomotor effect is still limited. This study was planned to investigate the vasoactive effects of fluoxetine on smooth muscle in rat thoracic aortic rings in an experimental setup.

Materials and Methods: 24 adult Wistar albino rats were divided into two groups. Group1-Endothelium intact group, Group2-Endothelium damaged group. Descending thoracic aorta was isolated after cervical dislocation. The aorta rings were immediately placed in organ bath chambers containing Krebs solution. Changes in isometric tension of aorta rings were recorded. Phenylephrine 10^{-6}M was administered and contractions were recorded in groups. Then, fluoxetine was given to Group 1 in cumulative doses (0.01, 0.1, 1, 2 mM). Endothelial damage was created in Group 2. After controlling the endothelial damage by acetylcholine 10^{-6}M, rings were washed for an hour and a second dose of phenylephrine was administered and then fluoxetine was given cumulatively to Group 2 and contractions were recorded.

Results: While the dose-dependent main vasodilator effect of fluoxetine was significantly different [F (5.110) =72.740, p<0.001, ηp2=0.77], the dose-group interaction was similar. After 1 mM administration of fluoxetine, less relaxation response occurred in Group 2.

Conclusion: The findings suggest that fluoxetine may have beneficial effects such as increasing blood flow on treatment of cardiovascular diseases.

Keywords:. Fluoxetine, rat, thoracic aorta, contraction, relaxation

Öz

Amaç: Literatürdeki çalışmaların çoğu fluoksetinin kardiyo/serebrovasküler sistemler üzerindeki etkilerine odaklanmış olsa da, vazomotor etkisi hakkında bilinenler hala sınırlıdır. Bu çalışma, fluoksetinin sıçan torasik aort halkalarında düz kas üzerindeki vazoaktif etkilerini deneysel bir düzende araştırmak için planlanmıştır.

Gereç ve Yöntem: 24 adet yetişkin Wistar albino rat iki gruba ayrıldı. Grup1-Endotel sağlam grup, Grup2-Endotel hasarlı grup. Servikal dislokasyon sonrası torasik aort izole edildi. Aort halkaları hemen Krebs solüsyonu içeren organ banyosu haznelerine yerleştirildi. Aort halkalarının izometrik gerimindeki değişiklikler kaydedildi. Fenilefrin 10^{-6}M uygulandı ve kasılmalar kaydedildi. Daha sonra Grup 1'e kümülatif dozlarda (0.01, 0.1, 1, 2 mM) fluoksetin uygulandı. Grup 2'de endotel hasarı oluşturuldu. Asetilkolin 10^{-6}M ile endotel hasarı kontrol edildikten sonra, halkalar bir saat yıkanarak ikinci doz fenilefrin hazneye eklendi. Ardından Grup 2'ye kümülatif olarak fluoksetin uygulanıp kasılmalar kaydedildi.

Bulgular: Fluoksetinin doza bağımlı ana vazodilatör etkisi anlamlı olarak farklıyken [F (5.110) =72.740, p<0.001, ηp2=0.77], doz-grup etkileşimi benzerdi. 1 mM fluoksetin uygulamasından sonra Grup 2'de daha az gevşeme yanıtı oluştu.

Sonuç: Bulgular fluoksetinin kardiyovasküler hastalıkların tedavisinde kan akımını artırma gibi faydalı etkileri olabileceğini düşündürmektedir.

Anahtar kelimeler: Fluoksetin, sıçan, torasik aort, kasılma, gevşeme

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INTRODUCTION

Depression is defined as an independent risk factor for the development of coronary artery disease (CAD). The lifetime prevalence of depression, which has many negative effects on the cardiovascular system, is almost one-fifth[1]. Decreased heart rate changes, abnormal platelet function, increased platelet reactivity, hypercortisolemia and endothelial dysfunction have an important place among the negative effects of depression on the cardiovascular system[1,2,3]. It is well known that there is a negative correlation between decreased serotonin levels and the development of depression[4]. Many serotonin reuptake inhibitor (SSRI) antidepressant drugs are used to correct functional disorders in the transfer and levels of serotonergic proteins[4,5,6,7]. SSRIs, which are widely used among antidepressant types, prevent serotonin reuptake by binding to serotonin reuptake sites in the synaptic area. Thus, in order to activate the postsynaptic neuron more, it allows serotonin to stay in the synaptic gap longer than normal[8].

Fluoxetine (Flu), the first available SSRI to enter clinical use in most countries, is an effective antidepressant agent[9,10]. The main active metabolite of norfluoxetine in the body, has by far the highest volume of distribution and a long half-life (1-4 days) among the SSRIs[10]. It is also known that fluoxetine can block nicotinic acetylcholine receptors[11], sodium [Na^+] [12], voltage-dependent potassium [K^+] [13] and calcium [Ca^{2+}] channels[14] in neuronal tissues, independently of its ability to inhibit neuronal serotonin reuptake. Moreover, fluoxetine is an antidepressant that can affect smooth muscle functions by blocking K^+ channels in isolated intestinal smooth muscle cells[15] and Ca^{2+} channels in isolated pulmonary arterial smooth muscle cells[16]. Many SSRI drugs, including fluoxetine, inhibit increased platelet serotonin receptor number and increased calcium mobilization in platelets in depression. Thus, fluoxetine may also reduce the risk of myocardial infarction by inhibiting serotonin-mediated platelet activation and cause an increase in bleeding time[17].

Beyond its antidepressant effect, fluoxetine plays an immunoregulatory role in preventing CAD by suppressing the production of inflammatory cytokines such as INF-gamma and TNF-alpha, which increase in depression and trigger inflammation with their inflammatory effects, which play an important role in the development of CAD. Moreover, fluoxetine plays an active role in the treatment of heart rate changes, which are indicators of autonomic dysregulation[18]. Fluoxetine can also directly affect cardiac contractility and heart rate with its negative inotropic effect by inhibiting the functions of many receptors, most of which are directly related to the regulation of vasomotor tone, such as 5-HT_{2C}, 5-HT_3 and nicotinic receptors. It is also stated that it prolongs the QT interval with similar mechanisms and may cause arrhythmogenic effects such as tachycardia[19].

Although it is known that fluoxetine has many effects on the cardiovascular system, its vasomotor effects on the cardiovascular system have not been fully elucidated. With this study, we aimed to present a different perspective on the preference of fluoxetine in the selection of antidepressants in people with cardiovascular disease. The aim of this study was to investigate the vasoactive effects of fluoxetine on rat thoracic aorta smooth muscle in an experimental model. We aimed to determine the possible vasorelaxative potential of fluoxetine in isolated rat thoracic aorta with intact and mechanically damaged endothelial tissue vasoconstricted with phenylephrine.

MATERIALS AND METHODS

Animals and husbandry

All experimental protocols performed in this study were approved by the Local Ethics Committee of Animal Experiments, University of Necmettin Erbakan (HADYEK, protocol number 034/2021). Rats were obtained from the Experimental Medicine Research and Application Center of Necmettin Erbakan University (Konya, Turkey) for experimental use. The care and housing of the experimental animals were carried out also in the Experimental Medicine Research and Application Center of Necmettin Erbakan University.

The study was performed in the Physiology Smooth Muscle Laboratory of Meram Faculty of Medicine, Necmettin Erbakan University. Adult Wistar Albino rats, weighing 230–260 g were used for the current study. A total of 24 rats were randomly divided into 2 equal groups.

Group 1: n=12 (Endothelium Intact Group=EI)

Group 2: n=12 (Endothelium Damaged Group=ED)

Before starting the experimental study, rats were left for habituation and fed by the standard commercial rodent chow (Purina 5001 Rodent Laboratory Chow including crude protein min. 23%, crude fat min. 4.5%, fiber max. 6%, ash max. 8%, added minerals max. 2.5%) with free access to water ad libitum with a light/dark cycle of 12 h, at a temperature of 21±2°C. All the procedures were performed according to "Guide for the Care and Use of Laboratory Animals" (NIH US publication No. 85-23, revised 1985) recommendations.

Experimental design and procedure

The rats were sacrificed by cervical dislocation under moderate general anesthesia. After thoracotomy, the descending thoracic aortas distal to arcus were quickly isolated and placed in a petri dish filled with Krebs–Henseleit solution. The adhering perivascular fat and connective tissue was carefully removed and arterial segments were cut into 3 to 4mm long rings. The aorta rings were suspended on hooks by tying silk thread between two different hooks, one end inside the chamber and the other end outside the chamber in transverse plane in a 10mL organ bath chamber filled with Krebs-Henseleit solution [composed of (mM/L): NaCl 119; KCl4.70; $MgSO_4$ 1.50; KH_2PO_4 1.20; $CaCl_2$ 2.50; $NaHCO_3$ 25;Glucose 11; EDTA 0.03] under a resting tension of 1 g,maintained at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2, pH 7.4, at constant flow of 4 ml/min. Adequate care was taken to insert the hooks without damaging the tissues. Tissues were allowed to equilibrate for 1 hour, meanwhile the solution was changed every 15-min for new-fresh solution.

After a 60-min-stabilization period, isometric contraction was induced by alpha-1 adrenoceptor agonist, phenylephrine (PE 10^{-6}M), Changes in isometric tension of aorta rings were recorded using a four-channel force-displacement transducer (MAY IOBS 99 Isolated Tissue Bath Stand Set Integrated Tissue Bath System, Commat, Ankara, Turkey). Distilled water was circulated in the thermocirculator (MAYWBC 3044-PR Heating Circulator, Commat, Ankara, Turkey) on the outer walls of all the chambers of the isolated organ bath system with a double-wall structure and four chambers. This water was used to keep the Krebs solution in the chamber at the required temperature. The liquid-gas transport apparatus enabled the Krebs solution to circulate throughout the entire organ bath and the mixture to reach the hoppers.

Fluoxetine (Flu) was given to endothelium intact group (n=12 rats) in cumulative increasing doses (0.01, 0.1, 1, 2 mM). Endothelial damage was created by scratching the endothelium of the aortic tissues in endothelium damaged group with a needle tip. The endothelial damage controlled by applying Acetylcholine (Ach 10^{-6}M). The vascular endothelium was considered as damaged when the aortic rings showed relaxation ≤10%[20]. After controlling the endothelial damage by applying Ach 10^{-6}M, the damaged aortic rings were washed for 1 h to reduce the effect of anesthetic agents, and a second dose of phenylephrine (PE 10^{-6}M) was administered. Then, cumulative inceasing doses (0.01, 0.1, 1, 2 mM) of Flu were given to endothelium damaged group (n=12 rats) and contractions were recorded. Contraction or relaxation (vasomotor) responses of the aortic rings which were obtained with administrations were recorded. Contractions were recorded as frequency and tension (milligram) in the isolated organ bath system[20,21].

Drugs

Phenylephrine (PE), Acetylcholine (Ach) and Fluoxetine (Flu) (dissolved in distilled water) were used. Phenylephrine and Acetylcholine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fluoxetine was kindly providedby Abdi İbrahim Drug Industry (Istanbul, Turkey). Distilled water was determined to have no effects on phenylephrine-induced contractions.

Statistical analysis

The results were considered statistically significant at the $p<0.05$ level and all tests were two-tailed. Data in the text were presented as mean ± SEM (Standard Error of Mean). Before fluoxetine was administered, Paired t test was applied in the analysis of the data of the experimental setup for pairwise comparisons within the group (in the analysis of the data before fluoxetine administration, it was applied in pairwise ways throughout the group). After cumulative Fluoxetine administration, a two-way repeated measures analysis of variance (ANOVA) was performed to test the main effects corresponding to Groups (EI, ED Group) and their doses, as well as the interaction between the two. Bonferroni correction was performed in the post-hoc comparison of dose groups. These analyzes were performed using the JASP Team (2019). JASP (Version 0.11.1) [Computer software]. G*Power

software was used to calculate the sample size. In order to determine the sample size in the current study, the effect size Cohen's suggested moderate (f=0.30)22, α= 0.05 margin of error and 0.90 power values were taken as reference. The sample size obtained was calculated as 22 rats. In addition, 10% animals were added to compensate for possible animal and tissue losses. Thus, the sample size to be studied was determined as 24 rats.

RESULTS

The self contraction of the aortic ring of the endothelium intact group was at 1310.78 ± 80.91 recorded. As shown in the Table 1, after the endothelium intact aortic rings were contracted with PE 10^{-6}M, a peak contraction was recorded at 2306.7±177.69. The difference between self contraction and PE 10^{-6}M dose-induced peak contraction was statistically significant in endothelium intact group (p<0.001).

The change of contraction was approximately 76%. This contraction response that occurs with PE 10^{-6}M was considered adequate for peak contraction before fluoxetine administration in endothelium intact group (Figure 1). Then, cumulative increasing doses of fluoxetine (0.01, 0.1, 1, 2 mM) was administered to the endothelium intact aortic rings in every 15 min, 15 minutes after PE 10^{-6}M was administered to the isolated organ bath chambers. As shown in Figure 2, isometric tensions were recorded as milligram.

Table 1. Tension values at measurement points in endothelium intact and endothelium damaged group

	EI Group (Tension-mg)	**ED Group** (Tension-mg)	**Within Groups F(5,110)**	**Interaction F(5,110)**	**Between Groups F(1,22)**
SC Basic	-	1201.81 ± 93.28*	-	-	-
PE 10^{-6}M First	-	2111.47 ± 171.21†	-	-	-
Ach 10^{-6}M	-	2031.98 ± 164.35††	-	-	-
SC	1310.78 ± 80.91¶	1232.22 ± 88.13‡	**p<0.001**, η_p^2 =0.77	p=0.289, η_p^2=0.05	p= 0.923, η_p^2=0.00
PE 10^{-6}M	2306.74 ± 177.69	2199.1 ± 183.3			
Flu 0.01mM	2052.37 ± 167.94	2079.24 ± 184.78			
Flu 0.1mM	1866.49 ± 143.28	1861.29 ± 163.1			
Flu 1mM	1494.12 ± 128.29	1584.99 ± 146.27			
Flu 2mM	1208.92 ± 78.41	1389.76 ± 118.66			

Endothelium Intact (EI) Group: ¶SC-PE 10-6M: p<0.001; Endothelium Damaged (ED) Group: *SC Basic-PE 10-6M First: p<0.001; †PE 10-6M First-Ach 10-6M: p=0.02 ††Ach 10-6M-SC: p<0.001; ‡SC-PE 10-6M: p<0.001; SC Basic; Basic Self Contraction in groups PE 10-6M First; First dose of Phenylephrine administered to reach peak contraction; Ach10-6 M; Acetylcholine given to confirm the presence of endothelial damage in the ED group; SC: Self Contraction; Flu: Fluoxetine (mM; mili Molar); Interaction: Dose X Group Tension (mg): Contraction; Data were presented as the mean ± standard error of mean (SEM)

After the equilibration period, the endothelium damaged thoracic aortic rings were contracted via PE 10^{-6}M first. Endothelial damage was controlled with the administration of acetylcholine (Ach 10^{-6}M), then damaged aortic rings were washed for one hour to reduce the effect of anesthetic agents, and PE 10^{-6}M dose was administered at the end of this period for triggering spontaneous contractions. The basic self contraction of endothelial damaged aortic ring was recorded at 1201.81±93.28. After the aortic rings were contracted with PE 10^{-6}M, a peak contraction was recorded at 2111,47±171,21 (Table 1).

The difference between basic self and PE 10^{-6}M dose-induced peak-contraction was statistically significant in endothelium damaged group (p<0.001). The change of contraction was approximately 75%. This contraction response that occurs with the first PE 10^{-6}M was considered adequate for peak contraction before Ach administration in endothelium damaged group (Figure 1). The self contraction of endothelium damaged group was recorded at 2031.98 ± 164.35 after Ach 10^{-6}M administration (Table 1). There is statistically significant difference between first PE 10^{-6}M-

induced peak-contraction and Ach 10^{-6}M dose-induced contraction inhibition (p=0.02) (Figure 2).

To determine the presence of functional endothelium in the endothelial damaged aortic ring, a relaxation response of approximately 3.75% developed against Ach 10^{-6}M administration. The low relaxation response after Ach 10^{-6}M administration to PE-induced contraction has been accepted as an indicator of the development of endothelial damage to a large extent (≤ 10%)[20].

After the relaxation response caused by the effect of Ach 10^{-6}M, the relaxation response revealed by the washing effect was also statistically significant (p<0.001). With this 39.3% relaxation response after washing, a value close to the initial basic tension value was reached in the endothelial damaged aortic ring. In other words, it was thought that the effects of the drugs administered before were no longer effective in the aortic rings and the initial experimental setup for Flu administration was healthy.

After washing and returning to self contraction (1232.22 ± 88.13), the aortic rings were contracted with PE 10^{-6}M and a peak contraction was recorded at 2199.1 ± 183.3 (Figure 2). The difference between self contraction and PE 10^{-6}M induced peak contraction were statistically significant in endothelium damaged group (p<0.001). The change of the contraction was approximately 78.5%. As shown in Figure 1, this contraction response that occured with PE 10^{-6}M was considered adequate for peak contraction before fluoxetine administration in the endothelium damaged group. Then, cumulative increasing doses of fluoxetine (0.01, 0.1, 1, 2 mM) was administered to the endothelium damaged aortic rings in every 15 minutes. As shown in Figure 2, isometric tensions were recorded as milligram.

The main effect of fluoxetine on the cumulative dose-dependent relaxation response was significant [F (5,110) = 72,740, p<0.001, η_p^2 =0.77]. That is, the relaxation response that occurred depending on the cumulatively increasing dose of Fluoxetine administered showed similar changes in both the endothelium intact and endothelium damaged groups.

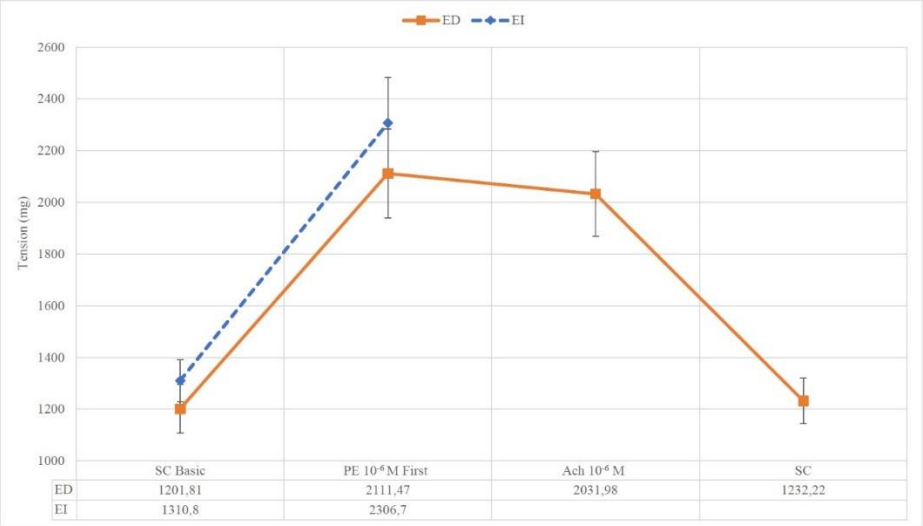
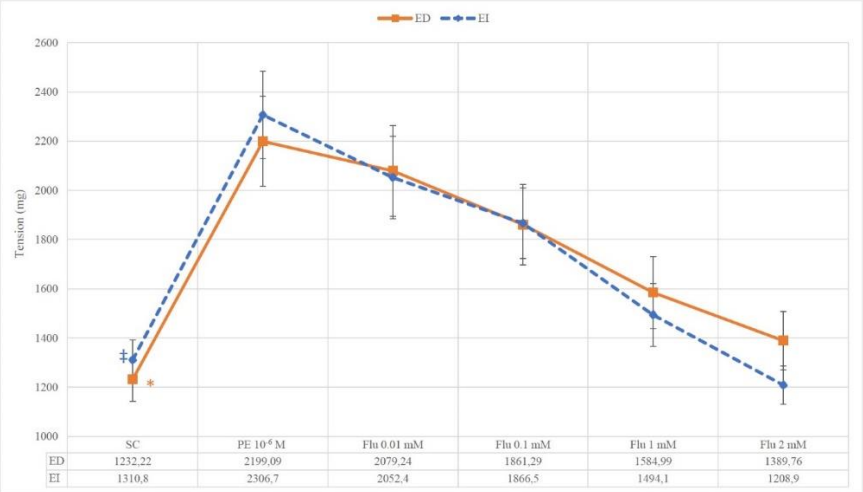


Figure 1. Administrations and results to create the experimental setup until the cumulatively increasing dose of Fluoxetine was administered in the endothelium intact group (EI) and endothelium damaged group (ED)

SC Basic; Basic Self Contraction in groups; PE 10-6M First; First dose of Phenylephrine administered to reach peak contraction; Ach10-6 M; Acetylcholine given to confirm the presence of endothelial damage in the EDG; SC; Self Contraction ; Tension (mg): Contraction; Data were presented as the mean.



	SC	PE 10^{-6} M	Flu 0.01 mM	Flu 0.1 mM	Flu 1 mM	Flu 2 mM
ED	1232,22	2199,09	2079,24	1861,29	1584,99	1389,76
EI	1310,8	2306,7	2052,4	1866,5	1494,1	1208,9

Figure 2. Contraction inhibition (relaxation) responses after cumulative Fluoxetine administration.

SC; Self contraction value measured before peak contraction induced with PE 10-6 M in groups; ‡ SC; Basal self contraction of endothelium intact (EI) group; * SC; After washing self contraction of endothelium damaged (ED) group); PE 10-6M; Phenylephrine administered to reach peak contraction (M; Molar); Flu; Fluoxetine (mM; mili Molar); Tension (mg): Contraction; Data were presented as the mean

In self-contraction before fluoxetine administration, the contraction response with PE 10-6M administration was significant ($p<0.001$) (Figure 2). On the other hand, there was a statistically significant difference in relaxation response between the cumulatively increased doses of Fluoxetine after the PE 10-6M dose-induced peak contraction in terms of dose-dependent effect compared to the previous dose. In other words, the relaxation response between the PE 10-6M dose-induced peak contraction point and the Flu 0.01 mM dose administration point was significant ($p<0.001$). In addition, as shown in Figure 2, there was a statistically significant difference in relaxation response between the dose administration points of Flu 0.01 mM and Flu 0.1 mM, Flu 0.1 mM and Flu 1 mM, Flu 1 mM and Flu 2 mM ($p<0.001$). There was no statistically significant difference between self contraction and Flu 2 mM dose administration ($p>0.05$) because the relaxation response had almost returned to the self contraction tension value after the last dose of Flu 2 mM.

Although the main effect of fluoxetine on the dose-dependent relaxation response was significant, the interaction of dose and group was not significant [$F(5, 110) = 1.254$, $p=0.289$, $\eta_p^2=0.05$]. The relaxation change that occurred depending on the fluoxetine dose was observed similarly in both groups (Figure 2).

The relaxation response lines running parallel to each other in the graph exactly coincide with the finding that the interaction of dose and group is not significant (Figure 2). The mean of the relaxation response, which developed depending on the dose of Fluoxetine administered, was similar in the endothelium intact and endothelial damaged groups, and there was no statistically significant difference between the groups in terms of the mean relaxant effect of fluoxetine [$F(1, 22) =0.010$, $p=0.923$, $\eta_p^2=0.00$].

DISCUSSION

In the present study, the vasoactive effects of fluoxetine were investigated in thoracic aortic rings of rats with intact and damaged endothelium. This study demonstrated that cumulatively increasing fluoxetine dose significantly induced dose-dependant vasorelaxation in both EI and ED groups, predrug-stimulated with phenylephrine. This dilatation effect of fluoxetine also occurred independently of endothelium-derived dilator factors such as nitric oxide, because there was no significant difference between groups in the mean of the relaxation response.

Some studies have shown that the SSRI family reduces the risk of chronic heart disease and has positive effects on the cardiovascular system[19,23,24,25].

Fluoxetine[26] and sertraline[27,28], which are popular among SSRIs, are known to be effective and safe in the treatment of patients with depression before or after an acute coronary syndrome event such as myocardial infarction or unstable angina. Although the acute and chronic effects of fluoxetine on the central nervous system are well defined, its effects on the cardiovascular system are not yet fully known. The cardioprotective beneficial effects of SSRIs, including fluoxetine; can be summarized as an increase in heart rate parameters, decrease in platelet hyperactivity and vasodilation[29].

It is known that fluoxetine exerts its serotonin reuptake inhibitory effect by blocking some serotonin receptors such as 5-HT_{2C} and 5-HT_3, or by interfering with the signal transduction pathways of serotonin[30,31]. Fluoxetine causes vascular smooth muscle tone changes with its blocking effect on many ion channel functions such as nicotinic acetylcholine receptors[11,32], voltage-gated Na^+ and K^+ [12,13], Ca^{2+} [14, 16, 33] and Cl [34] channels.

In the studies on rat brain arterioles performed by Ungvari et al. in 1999 and on human saphenous vein grafts by Akıncı et al. in 2019, it was shown that the vascular relaxation effects of fluoxetine were independent of endothelium-derived dilator factors or potassium channel activation. It has been stated that fluoxetine exerts its relaxant effect independent of the endothelium by interfering with the calcium signaling mechanisms that provide contraction in vascular smooth muscle[35,36]. In the present study, a significant relaxation response was observed in the rat thoracic aorta with the effect of cumulatively administered fluoxetine on phenylephrine-induced spontaneous contractions. The observed relaxation response was similar in both groups, suggesting that relaxation is independent of endothelial-derived relaxation factors.

Pereira et al. in 2017, showed that chronic fluoxetine treatment in Wistar albino rats reduced vasoconstriction caused by the effect of phenylephrine. The researchers showed that fluoxetine treatment administered for 21 days had no effect on vasoconstriction caused by the electrical field stimulation effect. They showed that chronic administration of fluoxetine regulates vascular sympathetic adrenergic responses by affecting presynaptic mechanisms and causes an increase in the amount of noradrenaline in the synaptic gap by preventing noradrenaline reuptake. Therefore, they stated that there was a decrease in vasoconstriction, peripheral vascular resistance and orthostatic hypotension[19].

It has been shown that even with a certain single dose of Fluoxetine in chronic stroke patients, muscle activity is positively affected[36]. On the other hand, short-term fluoxetine treatment increased the baroreflex control of sympathetic nervous system activity[38]. It is known that fluoxetine causes relaxation by blocking the voltage-dependent L-type Ca^{2+} channels in the vascular smooth muscle, preventing the contraction caused by the pressure effect in the vessel. Moreover, SSRIs, including Fluoxetine, have an inhibitory effect on atherogenic processes, thrombus formation and vascular occlusion by modulating platelet functions[39].

These results suggest that fluoxetine could potentially affect thoracic aortic vascular tone and thus blood flow in vivo. However, further studies are needed to clarify the effect of fluoxetine on cerebral blood flow in patients with major depression[40]. However, our findings also suggest that the mechanisms of action of fluoxetine are more complex than currently available information.

Another remarkable point in the results of the current study is that at the measurement points after Fluoxetine administration, the mean relaxation response in both groups was very close to each other until 1mM was administered. However, after 1mM Flu was administered in cumulatively increasing doses, although there was no statistical difference between the groups, the relaxation response began to differ between the groups in the direction of being less in the endothelium damaged tissue. That is, the lines between the two groups began to diverge, and this distinction became more pronounced after 2 mM dosing of Flu (the relaxation response in the endothelium intact and endothelium damaged group at Flu 1mM and 2 mM administration points; 37% / 14.8%, 48% / 19.9 %).

That is, the damaged endothelium showed less relaxation response. This finding supported our idea that endothelial damage and endothelial-derived dilator factors such as nitric oxide and endothelin are ineffective.

Another remarkable point is that no significant difference was observed in terms of relaxation response at Flu 1mM and Flu 2 mM doses in the groups. This finding may emphasize the importance of dose-adjustment in evaluating the relaxant effect

of fluoxetine for future experimental studies on similar experimental studies.

There are a few limitations in our study. Initially, the use of female rats was a major limiting factor because of cyclic variations of endocrine profile. However, during the study, female rats were the only gender available in our single source of animal. Latter, we used the thoracic aorta which was a big conductive vessel, but we did not have experience with a cerebral vessel.

Further studies with both fluoxetine and different SSRIs will provide further clarification of the mechanisms. Finally, we hope to investigate the possible mechanisms of the relaxant effect of fluoxetine or other SSRIs at the cellular level in the future, which we could not investigate due to our limited possibilities.

Fluoxetine, one of the most popular SSRIs, showed significant vasorelaxative effect in isolated rat thoracic aorta with both intact and mechanically damaged endothelial tissue vasoconstricted by phenylephrine in an experimental setting. Therefore, we might speculate that fluoxetine increases coronary and peripheral blood flow in vivo, which may contribute to its previously described beneficial effects in the case of cardiovascular diseases associated with depression.

Yazar Katkıları: Çalışma konsepti/Tasarımı: ZISG; Veri toplama: AO, AE; Veri analizi ve yorumlama: ROK, ZISG, HS; Yazı taslağı: BI; İçeriğin eleştirel incelenmesi: ZISG; Son onay ve sorumluluk: BI, ROK, ZISG, HS, AO, AE; Teknik ve malzeme desteği: ROK, ZISG, HS; Süpervizyon: ROK, ZISG, HS; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışmada gerçekleştirilen tüm deney protokolleri Necmettin Erbakan Üniversitesi Yerel Hayvan Deneyleri Etik Kurulu tarafından onaylanmıştır (HADYEK, protokol numarası 034/2021).
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarların beyan edecek çıkar çatışması yoktur.
Finansal Destek: Yazarlar bu makalenin araştırılması veya yayınlanması için herhangi bir maddi destek almamışlardır.
Yazarın Notu: İstatistik uygulamalarına verdiği destekten dolayı Yrd. doç. Serkan Küçüktürk'e teşekkür ederiz.

Author Contributions: Concept/Design : ZISG; Data acquisition: AO, AE; Data analysis and interpretation: ROK, ZISG, HS; Drafting manuscript: BI; Critical revision of manuscript: ZISG; Final approval and accountability: BI, ROK, ZISG, HS, AO, AE; Technical or material support: ROK, ZISG, HS; Supervision: ROK, ZISG, HS; Securing funding (if available): n/a.
Ethical Approval: All experimental protocols performed in this study were approved by the Local Ethics Committee of Animal Experiments, University of Necmettin Erbakan (HADYEK, protocol number 034/2021).
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