British Journal of Pharmacology (1999) 127, 740–746
http://www.stockton-press.co.uk/bjp

Serotonin reuptake inhibitor, fluoxetine, dilates isolated skeletal muscle arterioles. Possible role of altered Ca^{2+} sensitivity

[1]Pal Pacher, [2]Zoltan Ungvari, [1]Valéria Kecskemeti & *[,2,3]Akos Koller

[1]Department of Pharmacology, Semmelweis University of Medicine, P.O.Box 370, H-1445 Budapest, Hungary; [2]Institute of Pathophysiology, Semmelweis University of Medicine, P.O.Box 370, H-1445 Budapest, Hungary; and [3]Department of Physiology, New York Medical College, Valhalla, New York 10595, U.S.A.

1 Inhibitors of serotonin reuptake in the central nervous system, such as fluoxetine, may also affect the function of vascular tissues. Thus, we investigated the effect of fluoxetine on the vasomotor responses of isolated, pressurized arterioles of rat gracilis muscle (98 ± 4 μm in diameter at 80 mmHg perfusion pressure).

2 We have found that increasing concentrations of fluoxetine dilated arterioles up to 155 ± 5 μm with an EC_{50} of $2.5 \pm 0.5 \times 10^{-6}$ M.

3 Removal of the endothelium, application of 4-aminopyridine (4-AP, an inhibitor of aminopyridine sensitive K^+ channels), or use of glibenclamide (an inhibitor of ATP-sensitive K^+ channels) did not affect the vasodilator response to fluoxetine.

4 In the presence of 10^{-6}, 2×10^{-6} or 10^{-5} M fluoxetine noradrenaline (NA, 10^{-9}–10^{-5} M) and 5-hydroxytryptamine (5-HT, 10^{-9}–10^{-5} M)-induced constrictions were significantly attenuated resulting in concentration-dependent parallel rightward shifts of their dose-response curves ($pA_2 = 6.1 \pm 0.1$ and 6.9 ± 0.1, respectively).

5 Increasing concentrations of Ca^{2+} (10^{-4}–3×10^{-2} M) elicited arteriolar constrictions (up to $\sim$30%), which were markedly reduced by 2×10^{-6} M fluoxetine, whereas 10^{-5} M fluoxetine practically abolished these responses.

6 In conclusion, fluoxetine elicits substantial dilations of isolated skeletal muscle arterioles, a response which is not mediated by 4-AP- and ATP-sensitive K^+ channels or endothelium-derived dilator factors. The findings that fluoxetine had a greater inhibitory effect on Ca^{2+} elicited constrictions than on responses to NA and 5-HT suggest that fluoxetine may inhibit Ca^{2+} channel(s) or interfere with the signal transduction by Ca^{2+} in the vascular smooth muscle cells.

Keywords: Fluoxetine (Prozac); dilatation; endothelium; arteriolar potassium channels; 5-hydroxytryptamine; Ca^{2+} sensitivity

Abbreviations: ACh, acetylcholine; 4-AP, 4-aminopyridine; ATP, adenosine triphosphate; DOCA, desoxycorticosterone acetate; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; Endo+, intact endothelium; Endo−, endothelium removal; Glib, glibenclamide; 5-HT, 5-hydroxytryptamine; PS, physiological salt; NA, noradrenaline; SNP, sodium nitroprusside; SSRI, selective serotonin reuptake inhibitor

Introduction

Inhibition of serotonin (5-HT) reuptake in the central nervous system by fluoxetine (Prozac) has been widely used in the treatment of various psychiatric disorders. It is believed that this effect is specific without affecting the function of other tissues. However, several recent studies suggested that fluoxetine may have other effects, apparently not related to the inhibition of neuronal 5-HT reuptake. Fluoxetine was found to be a potent antagonist at muscular and neuronal nicotinic acetylcholine receptors (Colunga *et al*., 1997) and inhibitor of neuronal Na^+ (Pancrazio *et al*., 1998) and voltage-dependent potassium (Tytgat *et al*., 1997) channels. Furthermore, fluoxetine reduced nitrendipine binding to dihydropyridine-sensitive neuronal L-type Ca^{2+} channels and attenuated intracellular Ca^{2+} transients in fura-2 loaded synaptosomes (Stauderman *et al*., 1992). It has been suggeted that fluoxetine inhibits potassium-induced 5-HT release by decreasing voltage dependent Ca^{2+}-entry into nerve terminals, similar to results obtained with paroxetine, another serotonin reuptake inhibitor (Stauderman *et al*., 1992). Fluoxetine also inhibited several types of voltage-gated K^+ channels in cultured human corneal and lens epithelial cells (Rae *et al*., 1995).

In isolated rat uterus fluoxetine inhibited the contraction induced by high K^+ (Velasco *et al*., 1997). In smooth muscle cells, low concentrations of fluoxetine (10^{-7}–10^{-5} M) decreased the delayed rectifier K^+ current, whereas at higher concentrations (10^{-4} M) fluoxetine increased the Ca^{2+}-activated K^+ current (Farrugia, 1996).

Interestingly, there is an increasing number of case reports describing atrial fibrillation, bradycardia (Buff *et al*., 1991; Friedman, 1991; Anderson & Compton, 1997) and syncope (Ellison *et al*., 1990; McAnally *et al*., 1992; Cherin *et al*., 1997; Livshits & Danenberg, 1997; Rich *et al*., 1998) associated with fluoxetine treatment. Recently a multicenter case-control study has shown that in the elderly the consumption of fluoxetine was significantly associated with an excess risk of syncope and orthostatic hypotension (Cherin *et al*., 1997). A significant blood pressure lowering effect of fluoxetine was reported in DOCA-hypertensive rats (Fuller *et al*., 1979). The authors suggested that a central action of fluoxetine on vasomotor centers may be responsible for the reduction of blood pressure, but the possible direct cardiac and/or vascular effects of fluoxetine were not excluded or determined.

*Author for correspondence at: Department of Physiology, New York Medical College, Valhalla, New York 10595, U.S.A.; E-mail: kolako@net.sote.hu

Other types of antidepressant agents such as tricyclic and tetracyclic antidepressants have well documented cardiovascular effects in patients without pre-existing cardiac disease (Glassman, 1984; Burckhardt *et al*., 1978; Giardina *et al*., 1979; Vohra *et al*., 1975; Boehnert & Lovejoy, 1985). One of the most common manifestations of such effects is postural hypotension, arising in part from α-adrenergic blockade (Hayes *et al*., 1977; Glassman *et al*., 1979).

The possible underlying mechanisms of cardiovascular effects of fluoxetine, a newer non-tricyclic antidepressant agent known to be a potent and specific inhibitor of serotonin reuptake (SSRI), are not known.

On the basis of these previous findings we hypothesized that fluoxetine may affect the tone of resistance arterioles thereby promoting hypotension. In order to avoid the masking effect of neural and hormonal regulation of peripheral resistance the present study was undertaken to characterize the effect of fluoxetine in arterioles isolated from rat gracilis muscle. Furthermore, we aimed to elucidate the possible role of endothelium, various K^+ channels and altered Ca^{2+} sensitivity in fluoxetine-induced vasomotor responses.

Methods

Isolation of arterioles

Experiments were conducted on isolated arterioles ($\sim$100 μm active and $\sim$150 μm passive diameter at 80 mmHg) of male Wistar rats (weighing 140–180 g) gracilis muscle, as described previously (Koller *et al*., 1995a; Sun *et al*., 1996). Briefly, rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}). The gracilis muscle was isolated from surrounding tissues, dissected out and placed in a silicone-lined Petri dish containing cold (0–4°C) physiological salt (PS) solution composed of (in mM): NaCl 110, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.0, KH_2PO_4 1.0, dextrose 10.0 and NaHCO 24.0 and was equilibrated with a gas mixture of 10% O_2 and 5% CO_2, balanced with nitrogen, at pH 7.4. Using microsurgical instruments and an operating microscope a segment, 1.5–2 mm in length of an arteriole running intramuscularly was isolated, and transferred to an organ chamber containing two glass micropipettes filled with PS solution. From a reservoir the vessel chamber (15 ml) was continuously supplied with PS solution at a rate of 40 ml min^{-1}. After the vessel had been mounted on the proximal micropipette and was secured with sutures, the perfusion pressure was raised to 20 mmHg to clear the clotted blood from the lumen. Then the other end of the vessel was mounted on the distal pipette. Both micropipettes were connected with silicone tubing to an adjustable PS solution-reservoir. Pressure on both sides was measured by an electromanometer. The perfusion pressure was slowly (approximately over 1 min) increased to 80 mmHg. The temperature was set at 37°C by a temperature controller (Grant Instruments, Great Britain) and the vessel was allowed to equilibrate for approximately 1 h.

Experimental protocols

Only those vessels which developed spontaneous tone in response to perfusion pressure were used and thus no vasoactive agent was added to the PS solution to establish arteriolar tone. After the equilibration period the diameter of arterioles was measured at 80 mmHg perfusion pressure under zero-flow conditions by videomicroscopy as described previously (Koller *et al*., 1995a; Koller & Huang, 1997; Sun *et al*., 1996). At the conclusion of each experiment, the bath solution was changed to a Ca^{2+} free PS solution, which contained sodium nitroprusside (SNP, 10^{-4} M) and EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 1.0 mM). The vessel was incubated for 10 min, and the maximum passive diameter at 80 mmHg pressure was obtained (passive diameter). The diameter was measured with a microangiometer and recorded with a chart recorder.

Responses to increasing concentration of fluoxetine (10^{-7}–10^{-5} M) were obtained before and after endothelium removal or in the presence of 10^{-6} M glibenclamide (an inhibitor of ATP-dependent K^+-channels, Castle *et al*., 1989) or 10^{-5} M 4-aminopyridine (an inhibitor of aminopyridine sensitive K^+-channels, Castle *et al*., 1989). The endothelium of arterioles was removed by perfusion of the vessel with air for $\sim$1 min at a perfusion pressure of 20 mmHg (Koller *et al*., 1995a). The arteriole was then perfused with PS solution to clear the debris. Then the perfusion pressure was raised to 80 mmHg for 30 min to establish a stable tone. The efficacy of endothelial denudation was ascertained by arteriolar responses to acetylcholine (ACh, 10^{-7} M, an endothelium-dependent agent), and SNP (10^{-7} M, an endothelium-independent agent) before and after the administration of the air bolus. The infusion of air resulted in loss of function of the endothelium, as indicated by the absence of dilation to ACh, whereas dilation to SNP remained intact.

Responses to increasing concentration of pinacidil (an opener of ATP-dependent K^+-channel; 10^{-8}–10^{-4} M) were obtained before and after incubation with glibenclamide (10^{-6}M; 30 min). Arteriolar responses were obtained to increasing concentrations of 4-AP (10^{-6}–3×10^{-4} M).

In a second series of experiments responses of arterioles to cumulative doses of noradrenaline (NA, 10^{-9}–10^{-5} M) and 5-hydroxytryptamine (5-HT, 10^{-9}–10^{-5} M) were obtained. Then the vessel was incubated with fluoxetine (10^{-6}, 2×10^{-6} or 10^{-5} M) for 5 min and vasoactive responses were reassessed. After removal of the endothelium a Ca^{2+} free PSS was used to superfuse the vessels until maximal dilation developed. Then changes in diameter in response to increasing concentrations of Ca^{2+} (10^{-4}–3×10^{-2} M) were assessed before and after preincubation with fluoxetine (2×10^{-6} and 10^{-5} M; for 5 min). The osmolality of the bathing fluid was adjusted to 300 mosm at every concentration of $CaCl_2$ administered. All drugs were added to the vessel chamber and final concentrations are reported in the text. After responses to each drug subsided, the system was flushed with PS solution.

Materials

Fluoxetine and pinacidil were obtained from Research Biochemicals International (RBI) and Sigma Co. (U.S.A.). All other salts and chemicals were obtained from Sigma-Aldrich Co. (U.S.A.) and were prepared on the day of the experiment. Pinacidil was dissolved immediately before use in a small volume of 0.01N HCl and was diluted in buffer. 10^{-2} M stock of glibenclamide was prepared by dissolving the substance in 30% w v^{-1} (2-hydroxypropyl)-β-cyclodextrin (Cyclolab R&D Ltd, Hungary), adding a small volume of 40% v v^{-1} ethanol and dissolving in buffer. Fluoxetine was dissolved in PS solution.

Data analysis

Dilations were expressed as a percentage of the maximal dilation of arterioles defined as the passive diameter at 80 mmHg perfusion pressure in Ca^{2+} free media containing 10^{-3} M EGTA and 10^{-4} M SNP. Constrictions were expressed

as a percentage of the baseline diameter. From the logarithmic regressions of the cumulative dose-response curves of vasoactive agents the 50% effective concentrations (EC_{50}) were calculated. Where the drug interaction was apparently competitive, the negative logarithm (pA_2) of the equilibrium dissociation constant (K_e) was calculated for the antagonist (Arunlakshana & Schild, 1959; Kosterlitz & Watt, 1968). The K_e was calculated from the equation $K_e = a/(DR-1)$ where 'a' is the molar concentration of antagonist and 'DR', the dose ratio, is the measure of the rightward shift of the agonist dose-response curve. In the graphical plots the arithmetic mean ± s.e.mean values were used, whereas for the other parameters (EC_{50}, pA_2) means and s.d. values were calculated. Statistical analyses were performed by analysis of variance followed by Student's *t*-test. *P* values less than 0.05 ($P<0.05$) were considered statistically significant.

Results

Arterioles isolated from rat gracilis muscle developed spontaneous, myogenic tone in response to increasing the perfusion pressure to 80 mmHg without the use of any vasoactive agents. The active inner diameter of arterioles was 98 ± 4 μm. The passive diameter of arterioles obtained in the same conditions but in the absence of Ca^{2+} (see Methods) was 155 ± 5 μm.

Arteriolar responses to fluoxetine: role of endothelium

Fluoxetine (10^{-6} to 10^{-5} M) elicited substantial dilations in arterioles with an EC_{50} of $2.5 \pm 0.5 \times 10^{-6}$ M in a dose-dependent manner (Figure 1). We have also found that removal of endothelium had no significant effect on dilator responses of arterioles to fluoxetine ($EC_{50} = 2.6 \pm 0.4 \times 10^{-6}$ M; Figure 1).

Role of potassium channels

Next, we tested the possible role of ATP- and 4-aminopyridine-sensitive K^+-channels in the vasodilator action of fluoxetine. First we demonstrated that these K^+-channels are present in the gracilis arterioles. The ATP-sensitive K^+ channel opener pinacidil dilated the arterioles (Figure 2B) with an EC_{50} of $1.1 \pm 0.3 \times 10^{-7}$ M. Glibenclamide in a concentration of 10^{-6} M caused an approximately 20 fold significant ($P<0.01$), parallel rightward shift of pinacidil dose-response curve without affecting the maximum response. The further significant ($P<0.01$) rightward shift by 10^{-5} M glibenclamide was non-parallel. From the parallel shift, assuming a competitive interaction, a pA_2 of 7.3 ± 0.2 was calculated. 4-aminopyridine (10^{-5}–10^{-3} M) constricted the gracilis arterioles in a concentration-dependent manner (Figure 2C). After preincubation and in the presence of the ATP-sensitive K^+ channel inhibitor glibenclamide (10^{-6} M) fluoxetine-induced dilations of arterioles did not change significantly ($EC_{50} = 2.9 \pm 0.5 \times 10^{-6}$ M; Figure 2A). Similarly, preincubation of arterioles with 4-aminopyridine (10^{-5} M) did not affect the fluoxetine dose-response curve ($EC_{50} = 2.3 \pm 0.5 \times 10^{-6}$ M; Figure 2A).

Arteriolar responses to noradrenaline and 5-hydroxytryptamine

Next, we aimed to test the hypothesis that fluoxetine may interact with noradrenaline (NA) or 5-hydroxytryptamine (5-HT) receptors and/or the signal cascades. Therefore we tested arteriolar responses to NA and 5-HT. Noradrenaline constricted arterioles in a concentration-dependent manner with an EC_{50} of $1.2 \pm 0.4 \times 10^{-6}$ M (Figure 3A). Fluoxetine (2×10^{-6} M) caused an approximately 4 fold significant ($P<0.05$), parallel rightward shift of NA dose-response curve without affecting the maximum response. From the parallel shift, a pA_2 of 6.1 ± 0.1 was calculated.

5-Hydroxytryptamine constricted the arterioles in a concentration-dependent manner with an EC_{50} of $1.1 \pm 0.3 \times 10^{-7}$ M (Figure 3B). Fluoxetine (10^{-6} M and 2×10^{-6} M) caused an approximately 10 and 20 fold, parallel rightward shift of 5-HT dose-response curve respectively, without affecting the maximum response. From the parallel shifts, a pA_2 of 6.9 ± 0.1 was calculated. The calculated slope of Schild regression was -1.1, indicating competitive antagonism. Fluoxetine in a higher concentration (10^{-5} M) markedly reduced responses to 5-HT (10^{-8}–10^{-5} M; $n=4$) and NA (10^{-8}–10^{-4} M; $n=4$) (Figure 3A and 3B, respectively).

Arteriolar responses to Ca^{2+}

In order to elucidate the possible role of altered Ca^{2+} sensitivity in fluoxetine-induced dilation we tested the arteriolar responses to increasing concentrations of calcium. In the absence of Ca^{2+} endothelium-denuded arterioles dilated maximally. Administration of Ca^{2+} (10^{-4}–3×10^{-2} M) in a dose-dependent manner elicited constrictions, restoring the myogenic tone of arterioles (developed in response to 80 mmHg perfusion pressure) (Figure 4). The dose-response curve to the increasing concentration of Ca^{2+} was characterized by a steep part indicating substantial constriction (10^{-4}–7.5×10^{-4} M) and a plateau phase (10^{-3}–3×10^{-2} M), where the arteriolar tone was maintained. The presence of 2×10^{-6} M and 10^{-5} M fluoxetine in the bath solution markedly ($P<0.01$) reduced Ca^{2+}-elicited constrictions compared to controls (Figure 4).

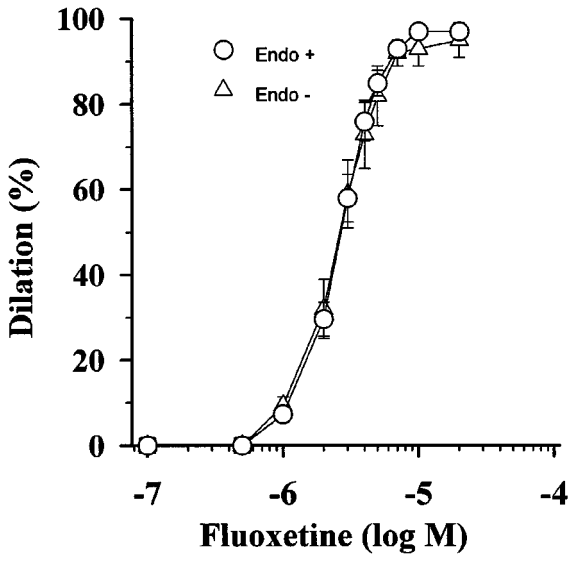

Figure 1 Effect of cumulative doses of fluoxetine on normalized diameter of arterioles isolated from rat gracilis muscle with intact endothelium (Endo+; $n=10$) and after the endothelium removal (Endo−; $n=7$). Data are mean ± s.e.mean.

Discussion

The main findings of our study are that the serotonin reuptake inhibitor fluoxetine dilates isolated rat skeletal muscle arterioles; this dilation is not mediated by 4-AP- or ATP-sensitive K^+ channels or endothelium-derived dilator factors; fluoxetine reduced arteriolar constrictions to NA and 5-HT in a competitive manner, and had a substantial inhibitory effect on Ca^{2+}-elicited constrictions.

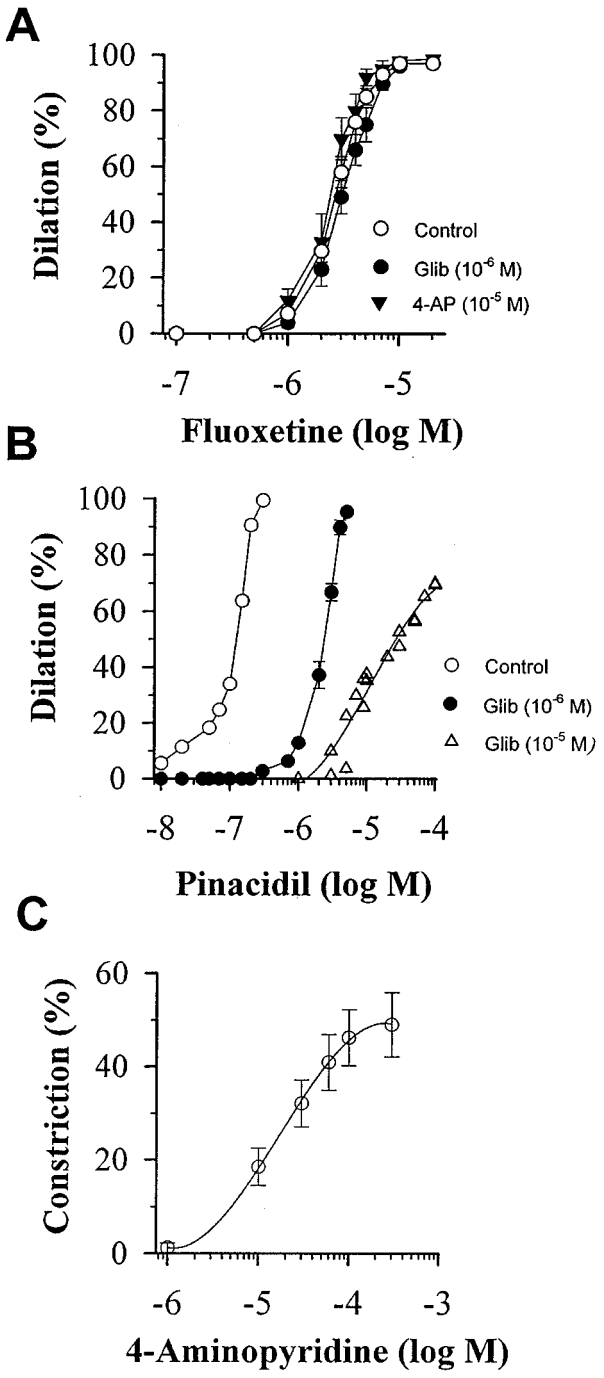

Figure 2 (A) Effect of cumulative doses of fluoxetine on normalized diameter of arterioles isolated from rat gracilis muscle in control conditions (Control, $n=10$), in the presence of 10^{-6} M glibenclamide (Glib, $n=5$), or 10^{-5} M 4-aminopyridine (4-AP, $n=5$). (B) Effect of cumulative doses of pinacidil in the absence (Control, $n=4$) or in the presence of 10^{-6} M ($n=4$) or 10^{-5} M ($n=2$) glibenclamide (Glib). (C) Effect of cumulative doses of 4-aminopyridine (4-AP) on normalized diameter of gracilis arterioles ($n=5$). Data are means ± s.e.mean.

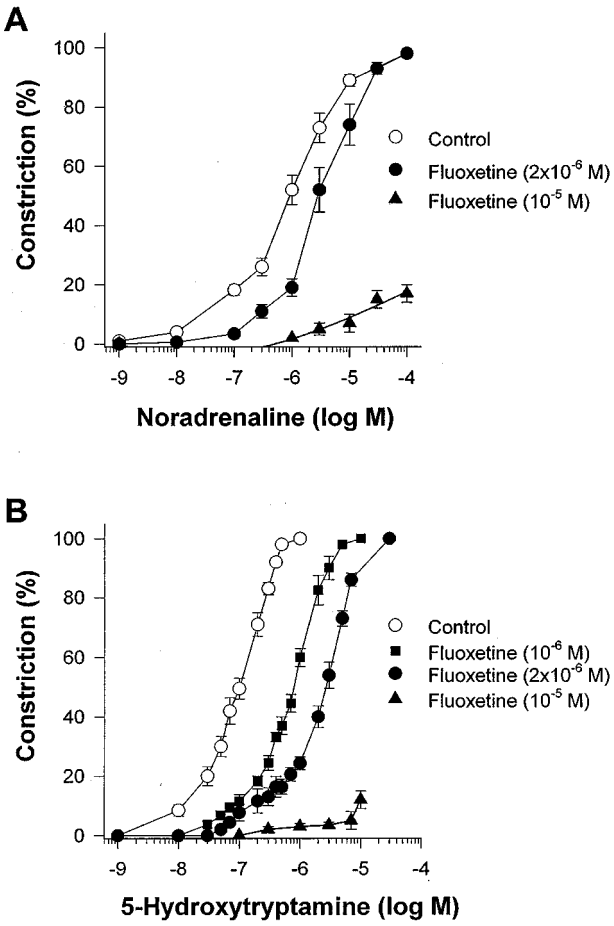

Figure 3 (A) Changes in normalized diameter of rat gracilis arterioles in response to cumulative doses of noradrenaline (NA) in the absence (Control, $n=6$) or presence of 2×10^{-6} M ($n=6$) or 10^{-5} M ($n=4$) fluoxetine. (B) Changes in diameter of rat gracilis arterioles in response to cumulative doses of 5-hydroxytryptamine (5-HT) in the absence (Control, $n=6$) or presence of 10^{-6} M ($n=6$), 2×10^{-6} M ($n=6$) or 10^{-5} M ($n=4$) fluoxetine. Data are means ± s.e.mean.

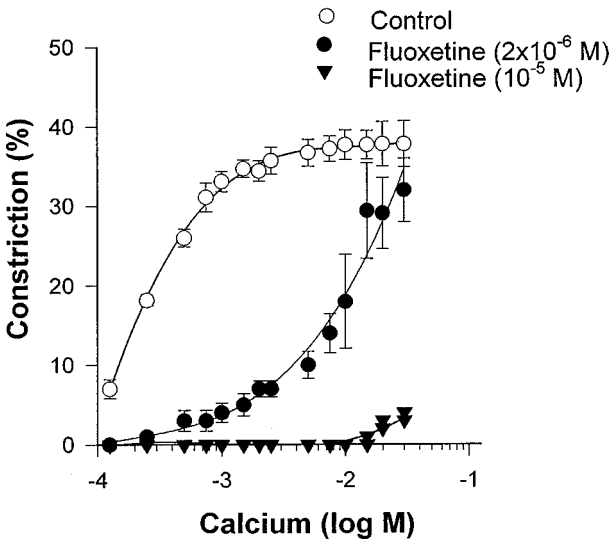

Figure 4 Changes in normalized diameter to increasing concentrations of calcium ($CaCl_2$) in the absence (Control, $n=8$) and in the presence of 2×10^{-6} M ($n=5$), or 10^{-5} M ($n=4$) fluoxetine. Data are means ± s.e.mean.

Although fluoxetine is regarded a specific inhibitor of serotonin reuptake in the neural tissue there are studies indicating that fluoxetine may have cardiovascular effects as well. There is an increasing number of case reports on bradycardia, dysrhythmia (Buff *et al*., 1991; Friedman, 1991; Anderson & Compton, 1997) and hypotensive events (Ellison *et al*., 1990; McAnally *et al*., 1992; Cherin *et al*., 1997; Livshits & Danenberg, 1997; Rich *et al*., 1998) associated with fluoxetine treatment. Interestingly in an early study the blood pressure lowering effect of fluoxetine has been described in DOCA-hypertensive rats (Fuller *et al*., 1979). The authors hypothesized that a central action of fluoxetine on vasomotor centre underlies this effect, but the possible direct cardiac and peripheral vascular effects of fluoxetine were not elucidated.

In the present study we hypothesized that fluoxetine may affect the tone of resistance arterioles thereby promoting vasodilation and hypotension. Thus we aimed to characterize the direct effect of fluoxetine on the vasomotor tone of isolated rat gracilis muscle arterioles.

Fluoxetine dilates skeletal muscle arterioles

We have demonstrated that fluoxetine has a concentration-dependent vasodilator action in skeletal muscle arterioles with an EC_{50} of $2.5 \pm 0.5 \times 10^{-6}$ M (Figure 1). These microvessels are representative of resistance vessels determining peripheral vascular resistance. The concentration of fluoxetine which has a substantial dilator effect falls into the upper range of therapeutic plasma concentrations ($0.15 - 1.5 \times 10^{-6}$ M) (Orsulak *et al*., 1988; Kelly *et al*., 1989; Pato *et al*., 1991). Previous studies reported similar concentrations of norfluoxetine, the active metabolite of fluoxetine also present in the plasma of fluoxetine-treated patients (Orsulak *et al*., 1988; Kelly *et al*., 1989; Pato *et al*., 1991). Furthermore, under certain conditions (e.g. decreased metabolism in elderly, acute overdose etc) the plasma concentration of fluoxetine and norfluoxetine can reach even higher levels (Pato *et al*., 1991; Borys *et al*., 1992; Hale, 1993). In addition, it is possible that fluoxetine can be accumulated in various tissues. During chronic fluoxetine treatment a concentration of fluoxetine 20 times higher than the plasma has been detected in human brain (Karson *et al*., 1993; Komoroski *et al*., 1994). Collectively, these findings suggest that under certain conditions the concentration of fluoxetine could reach levels which may affect peripheral vascular tone.

Possible mechanisms of action: role of endothelium

Removal of the endothelium did not affect fluoxetine induced dilations (Figure 1). Therefore we conclude that fluoxetine exerts its effect directly on the arteriolar smooth muscle cells without endothelial mediation.

Role of K^+ channels

As earlier studies suggested that fluoxetine may affect various K^+ channels in cells, we hypothesized that opening of arteriolar K^+ channels may play a role in the vasodilator action of fluoxetine. First we demonstrated the presence of the ATP-sensitive K^+ channels and the 4-AP-sensitive K^+ channels on the gracilis arteriole (Figure 2). Pinacidil, a known ATP-sensitive K^+ channel opener dilated the arterioles and this dilation could be competitively antagonized with glibenclamide, a specific inhibitor of this channel type. The non-selective inhibitor of various K^+ channels, 4-AP elicited significant constriction of the gracilis arterioles indicating that these channels are functional in the regulation of basal arteriolar tone. However, neither the inhibition of the ATP-sensitive K^+ channels, nor the 4-AP sensitive K^+ channels significantly affected the vasodilator action of fluoxetine (Figure 2).

Role of NA/5-HT receptor inhibition

It is well documented that tricyclic antidepressant drugs act on 5-HT and NA receptors both in the central nervous system and in the cardiovascular system (Baldessarini, 1997). Fluoxetine is also known to interfere with 5-HT receptors and/or signal transduction pathways in central nervous system (Fan, 1994; Ni & Miledi, 1997). However, less is known about the possible interaction between fluoxetine and 5-HT and NA receptors in vascular tissue. Similarly to previous studies (Koller *et al*. 1995b), NA and 5-HT elicited substantial constrictions of gracilis muscle arterioles. In the present study we found that fluoxetine caused a parallel rightward shift both in the 5-HT and NA dose-response curves in skeletal muscle arterioles. On the basis of these data one can assume, that fluoxetine antagonizes 5-HT and NA receptors in resistance arterioles.

Role of Ca^{2+}-sensitivity

It is known that an increase in intracellular Ca^{2+} in the arteriolar smooth muscle follows the stimulation of various NA and 5-HT receptors and is also necessary for maintaining myogenic arteriolar tone. It is well documentated (Hill & Meininger, 1994; Koller *et al*., 1995a; Sun *et al*., 1996; Koller & Huang, 1997) that arteriolar myogenic constriction which develops in response to increases in intraluminal pressure depends on the activity of Ca^{2+} channels in the vascular smooth muscle cells. In the absence of Ca^{2+}, arterioles dilated maximally, subsequent administration of increasing doses of Ca^{2+} elicited substantial constriction. Thus, at 80 mmHg perfusion pressure we studied the magnitude of Ca^{2+}-induced myogenic tone in the presence of fluoxetine (2×10^{-6} M), which markedly reduced calcium-induced vasoconstriction (a $\sim$50 fold rightward shift of Ca^{2+} dose-response curve), whereas a higher concentration of fluoxetine (10^{-5} mol^{-1} L) practically abolished Ca^{2+}-induced responses. Comparison of data indicates that fluoxetine reduces arteriolar Ca^{2+} sensitivity more effectively than the constrictor responses to NA or 5-HT. Therefore it is likely that fluoxetine elicits dilation by inhibiting Ca^{2+} entry to vascular smooth muscle cell. Previous studies support this hypothesis, demonstrating that fluoxetine antagonizes neuronal Ca^{2+} channels (Stauderman *et al*., 1992; Lavoie *et al*., 1997). Furthermore, we have recently found that fluoxetine caused a concentration-dependent decrease in contractile force and depression of the amplitude, overshoot and maximum rate of rise of depolarization of action potential in right ventricular papillary muscles of the rat (Pacher *et al*., 1998).

Collectively, these findings support the hypothesis that fluoxetine by inhibiting Ca^{2+} channel activity and/or altering Ca^{2+} sensitivity of smooth muscle cell, elicits an endothelium-independent arteriolar dilation. The fluoxetine induced reduction in arteriolar tone may underlie the hypotensive events occasionally observed during fluoxetine administration. It is also interesting to note that several vasorelaxant Ca^{2+} channel antagonists, especially dihydropyridine derivatives, were found to exert certain antidepressant effects (Nowak *et al*., 1993). Future studies should determine the possible involvement of inhibition of vascular and/or neural Ca^{2+} channels in the antidepressant action of fluoxetine.

In conclusion we found that fluoxetine elicits substantial dilation of isolated skeletal muscle arterioles. This response is not mediated by 4-AP- or ATP-sensitive K^+ channels or endothelium-derived dilator factors. Our results suggest that fluoxetine may antagonize adrenergic and serotinergic receptors and/or block either entry of Ca^{2+} or may interfere with the Ca^{2+}-signal transduction in the arteriolar smooth muscle cells, favouring reduction of vascular resistance.

We thank Drs Andras Ronai and Peter Rajna for their advice of great value. This work was supported by Grants from the Hungarian National Science Research Foundation OTKA T-023863, OTKA T-019206, the Health Science Council ETT 524/97 and the National Heart, Lung and Blood Institute U.S.A. HL-46813.

References

ANDERSON, J. & COMPTON, S.A. (1997). Fluoxetine induced bradycardia in presenile dementia. *Ulster Med. J.,* **66,** 144–145.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.,* **14,** 48–58.

BALDESSARINI, R.J. (1997). Drugs and the treatment of psychiatric disorders. In *The pharmacological basis of therapeutics,* 9th edition. eds. Hardman, J.G; Limbird, L.E.; Molinoff, P.B.; Ruddon, R.W. & Gilman, A.G. pp 431–460. New York: McGraw-Hill.

BOEHNERT, M.T. & LOVEJOY JR. F.H. (1985). Value of the QRS duration versus the serun drug level in predicting seizures and ventricular arrhythmias after an acute overdose of tricyclic antidepressants. *N. Engl. J. Med.,* **313,** 474–479.

BORYS, D.J., SETZER, S.C., LING, L.J., REISDORF, J.J., DAY, L.C. & KRENZELOK, E.P. (1992). Acute fluoxetine overdose: a report of 234 cases. *Am. J. Emerg. Med.,* **10,** 115–120.

BUFF, D.D., BRENNER, R., KIRTANE, S.S. & GILBOA, R. (1991). Dysrhythmia associated with fluoxetine treatment in elderly patients with cardiac disease. *J. Clin. Psychiatry.,* **52,** 174–176.

BURCKHARDT, D., RAEDER, E., MULLER, V., IMHOF, P. & NEUBAUER, H. (1978). Cardiovascular effects of tricyclic and tetracyclic antidepressants. *J.A.M.A.,* **239,** 213–216.

CASTLE, N.A., HAYLETT, D.G. & JENKINSON, D.H. (1989). Toxins in the characterization of potassium channels. *T.I.N.S.,* **12,** 59–65.

CHERIN, P., COLVEZ, A., DEVILLE, DE PERIERE, G. & SERENI, D. (1997). Risk of syncope in the elderly and consumption of drugs: a case-control study. *J. Clin. Epidemiol.,* **50,** 313–320.

COLUNGA, G.J., AWAD, J.N. & MILEDI, R. (1997). Blockage of muscle and neuronal nicotinic acetylcholine receptors by fluoxetine (Prozac). *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 2041–2044.

ELLISON, J.M., MILOFSKY, J.E. & ELY, E. (1990). Fluoxetine-induced bradycardia and syncope in two patients. *J. Clin. Psychiatry,* **51,** 385–386.

FAN, P. (1994). Inhibition of a 5-HT3 receptor-mediated current by the selective serotonin uptake inhibitor, fluoxetine. *Neurosci. Lett.,* **173,** 210–212.

FARRUGIA, G. (1996). Modulation of ionic currents in isolated canine and human jejunal circular smooth muscle cells by fluoxetine. *Gastroenterology,* **110,** 1438–1445.

FRIEDMAN, E.H. (1991). Fluoxetine-induced bradycardia. *J. Clin. Psychiatry.,* **52,** 174–176.

FULLER, R.W., HOLLAND, D.R., YEN, T.T., BEMIS, K.G. & STAMM, N.B. (1979). Antihypertensive effects of fluoxetine and L-5-hydroxytryptophan in rats. *Life Sci.,* **25,** 1237–1242.

GIARDINA, E.G., BIGGER, JR, J.T., GLASSMAN, A.H. & PEREL, J.M., KANTOR, S.J. (1979). The electrocardiographic and antiarrhytmic effects of imipramine hydroclorid at therapeutic plasma concentrations. *Circulation,* **60,** 1045–1052.

GLASSMAN, A.H. (1984). Cardiovascular effects of tricyclic antidepressants. *Annu. Rev. Med.,* **35,** 503–511.

GLASSMAN, A.H., BIGGER JR. J.T., GIARDINA, E.V., KANTOR, S.J., PEREL, J.M. & DAVIES, M. (1979). Clinical characteristics of imipramine-induced orthostatic hypotension. *Lancet,* **1,** 468–472.

HALE, A.S. (1993). New antidepressants: use in high-risk patients. *J. Clin. Psychiatry,* **54,** 61–70.

HAYES, J.R., BORN, J.F. & ROSENBAUM, A.H. (1977). Incidence of orthostatic hypotension in patients with primary affective disorders treated with tricyclic antidepressants. *Mayo Clin. Proc.,* **52,** 509–512.

HILL, M.A. & MEININGER, G.A. (1994). Calcium entry and myogenic phenomena in skeletal muscle arterioles. *Am. J. Physiol.,* **267,** H1085–H1092.

KARSON, C.N., NEWTON, J.E., LIVINGSTON, R., JOLLY, J.B., COOPER, T.B., SPRIGG, J. & KOMOROSKI, R.A. (1993). Human brain fluoxetine concentrations. *J. Neuropsychiatry Clin. Neurosci.,* **5,** 322–329.

KELLY, M.W., PERRY, P.J., HOLSTAD, S.G. & GARVEY, M.J. (1989). Serum fluoxetine and norfluoxetine concentrations and antidepressant response. *The Drug Monit.,* **11,** 165–170.

KOLLER, A. & HUANG, A. (1997). Endothelin and prostaglandin H_2 enhance arteriolar myogenic tone in hypertension. *Hypertension,* **30,** 1210–1215.

KOLLER, A., HUANG, A., SUN, D. & KALEY, G. (1995a). Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles. Role of endothelial nitric oxide and prostaglandins. *Circ. Res.,* **76,** 544–550.

KOLLER, A., RODENBURG, J.M. & KALEY, G. (1995b). Effects of Hoe-140 and ramiprilat on arteriolar tone and dilation to bradykinin in skeletal muscle of rats. *Am. J. Physiol.,* **268,** H1628–H1633.

KOMOROSKI, R.A., NEWTON, J.E., CARDWELL, D., SPRIGG, J., PEARCE, J. & KARSON, C.N. (1994). In vivo 19F spin relaxation and localized spectroscopy of fluoxetine in human brain. *Magn. Reson. Med.,* **31,** 204–211.

KOSTERLITZ, H.W. & WATT, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists, with particular reference to N-allylnoroxymorphone (naloxone). *Br. J. Pharmacol.,* **33,** 266–276.

LAVOIE, P.A., BEAUCHAMP, G. & ELIE, R. (1997). Atypical antidepressants inhibit depolarization-induced calcium uptake in rat hippocampus synaptosomes. *Can. J. Physiol. Pharmacol.,* **75,** 983–987.

LIVSHITS, A.T.L. & DANENBERG, H.D. (1997). Tachycardia, orthostatic hypotension and profound weakness due to concomitant use of fluoxetine and nifedipine. *Pharmacopsychiatry,* **30,** 274–275.

MCANALLY, L.E., THRELKELD, K.R. & DREYLING, C.A. (1992). Case report of a syncopal episode associated with fluoxetine. *Ann. Pharmacother.,* **26,** 1090–1091.

NI, Y.G. & MILEDI, R. (1997). Blockage of 5HT2C serotonin receptors by fluoxetine (Prozac). *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 2036–2040.

NOWAK, G., PAUL, I.A., POPIK, P., YOUNG. A. & SKOLNICK, P. (1993). Ca^{2+} antagonists effect an antidepressant-like adaptation of the NMDA receptor complex. *Eur. J. Pharmacol.,* **247,** 101–102.

ORSULAK, P.J., KENNEY, J.T., DEBUS, J.R., CROWLEY, G. & WITTMAN, P.D. (1988). Determination of the antidepressant fluoxetine and its metabolite norfluoxetine in serum by reversed-phase HPLC, with ultraviolet detection. *Clin. Chem.,* **34,** 1875–1878.

PACHER, P., KECSKEMETI, V., UNGVARI, Z., KOROM, S. & PANKUCSI, C. (1998). Cardiodepressant effects of fluoxetine in isolated heart preparations. *Naunyn-Schmideberg's Arch. Pharmacol.,* **358** (suppl 2): R641.

PANCRAZIO, J.J., KAMATCHI, G.L., ROSCOE, A.K. & LYNCH, C. (1998). Inhibition of neuronal Na^+ channels by antidepressant drugs. *J. Pharmacol. Exp. Ther.,* **284,** 208–214.

PATO, M.T., MURPHY, D.L., & DEVANE, C.L (1991). Sustained plasma concentrations of fluoxetine/or norfluoxetine four or eight weeks after fluoxetine discontinuation. *J. Clin. Psychopharmacol.,* **11,** 224–225.

RAE, J.L., RICH, A., ZAMUDIO, A.C. & CANDIA, O.A. (1995). Effect of Prozac on whole cell ionic currents in lens and corneal epithelia. *Am. J. Physiol.,* **269,** C250–C256.

RICH, J.M., NJO, L., ROBERTS, K.W. & SMITH, K.P. (1998). Unusual hypotension and bradycardia in a patient receiving fenfluramine, phentermine and fluoxetine. *Anesthesiology,* **88,** 529–531.

STAUDERMAN, K.A., GANDHI, V.C. & JONES, D.J. (1992). Fluoxetine-induced inhibition of synaptosomal (^{3}H)5-HT release: possible Ca^{2+}-channel inhibition. *Life Sci.,* **50,** 2125–2138.

SUN, D., KALEY, G. & KOLLER, A. (1996). Characteristics and origin of myogenic response in isolated gracilis muscle arterioles. *Am. J. Physiol.,* **266,** H1177–H1183.

TYTGAT, J., MAERTENS, C. & DAENENS, P. (1997). Effect of fluoxetine on a neuronal, voltage-dependent potassium channel (Kv1.1). *Br. J. Pharmacol.,* **122,** 1417–1424.

VELASCO, A., ALAMO, C., HERVAS, J. & CARVAJAL, A. (1997). Effects of fluoxetine hydrochloride and fluvoxamine maleate on different preparations of isolated guinea-pig and rat organ tissues. *Gen. Pharmacol.,* **28,** 509–512.

VOHRA, J., BURROWS, G., HUNT, D. & SLOMAN, G. (1975). The effects of toxic and therapeutic doses of tricyclic antidepressant drugs on intracardiac conduction. *Eur. J. Cardiol.,* **3,** 219–227.

(Received December 9, 1998
Revised February 25, 1999
Accepted March 1, 1999)