

Fluoxetine causes decrease in intestinal motility

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ABSTRACT

Background: Major depression is the most frequent disorder occurring in 16% of the population worldwide. In the middle of the 20th century, the discovery of selective serotonin (5-HT) reuptake inhibitors acted as a miracle in the antidepressant therapy. We explored the acute effects of fluoxetine and possible mechanism underlying the contractile effects of fluoxetine on isolated ileal smooth muscles of rabbit *in vitro*.

Methods: Effects of increasing concentrations of acetylcholine (Ach), 5-HT and fluoxetine were studied on isolated ileal tissue of the rabbit *in vitro* by constructing cumulative concentration response curves. The ileal smooth muscle contractions were recorded on power lab (USA).

Results: Ach, 5-HT and fluoxetine, produced a concentration-dependent reversible contraction of isolated ileal muscle of rabbit. The mean \pm standard error of the mean of maximum amplitudes of contraction with Ach, 5-HT and fluoxetine, were 24.8 ± 1.22 mm, 44 ± 0.527 mm and 2.6 ± 1.16 mm, respectively. Fluoxetine shifted the concentration-response curve right and downwards.

Conclusion: Our study has indicated that fluoxetine on isolated ileal intestinal smooth muscle decrease the motility and this decrease in motility is possibly due to the inability of fluoxetine *in vitro* to enhance the serotonergic transmission and also because of the interaction of these agents with some of the other receptors, present in the intestinal smooth muscles.

Keywords: Serotonin, Fluoxetine, Acetylcholine, Gastrointestinal tract, Diarrhea

INTRODUCTION

Major depression is the most frequent disorder occurring in sixteen percent of the population worldwide. It is becoming a major public health problem as it leads to depression induced disability, miserable lifestyle, the economic burden and in severe cases can lead to increase in suicidal tendencies. Developments of antidepressants in the last five decades were based on the monoaminergic hypothesis, which postulates that depression is because of deficiencies or fluctuation in the levels of serotonin (5-HT), nor-epinephrine and dopamine. The antidepressants act by improving the neuroplasticity via increasing the expression of brain-derived neurotrophic factors, but it takes weeks to months for therapeutic effects to appear.¹

First Fluoxetine was introduced. Selective 5-HT reuptake inhibitors not only improve the efficacy but also reduce the time span for the appearance of therapeutic effects. Although

its untoward effects are less and are well-tolerated, but one of the most common gastrointestinal adverse effect is diarrhea.² The present study has been designed to observe the effects of fluoxetine on gastrointestinal smooth muscle of rabbits. 5-HT is the basic neurotransmitter in the gastrointestinal tract, however, its release is dependent on acetylcholine (Ach). First Ach is released which in turn stimulates the release of Ach.³ Hence to evaluate the extent of fluoxetine-induced intestinal activity, Ach-mediated intestinal activity was taken as standard (control) in our research study.⁴

METHODS

This experimental study was carried out in Pharmacology Department in collaboration with Centre for Research in Experimental and Applied Medicine Army Medical College Rawalpindi, from December 2012 to July 2013.

Chemicals/drugs

Ach chloride (Sigma Chemical Co., USA), 5-HT Carnitine Sulfate (Sigma Chemical Co., USA) and Fluoxetine Hydrochloride (Werrick Pharmaceuticals) were purchased. All the solutions and dilutions (10^{-9} - 10^{-6} M) were prepared fresh in distilled water at the time of experiments.⁵

Preparation of tissue

A total of 18 rabbits were randomly divided into three groups (n=6) after the approval of Ethics Committee of CREAM. Overnight fasting rabbit was sacrificed. The small intestine was taken out by recognizing the cecum and cut down into two inch pieces.⁶ The intestinal tissue was thoroughly washed with normal saline, and fecal content was removed, fatty tissues dissected. The isolated tissue was then transferred to isolated organ bath of 50 ml capacity containing tyrode's solution (in mM: NaCl, 136.8 mM; KCl, 2.7 mM; MgCl₂, 0.5 mM; CaCl₂, 1.3 mM; NaH₂PO₄, 0.14 mM; NaHCO₃, 12.0 mM, Dextrose, 5.5 mM) and aerated continuously with 95% oxygen and 5% carbondioxide.⁵ The cumulative dose response curve was made using power lab (USA). One end of the ileal strip was attached to the bottom of the oxygen tube in the tissue bath, and the other end was connected to a research grade force displacement transducer DT-475 (USA) by means of a thread. iWorx/214 (USA) was connected to the computer. Plug the DIN connector on the cable of the DT-475 displacement transducer into channel 3 of the iWorx/214 unit. The tissue was allowed a period of equilibration of 15 mins and physiological solution was changed 2 times.⁷ The isotonic ileal smooth muscle activity was recorded through the displacement transducer.⁸

Group 1: Cumulative concentration response curve of Ach (n=6)

Cumulative dose-response curves of Ach were constructed with varying concentrations (10^{-9} - 10^{-6} M). When maximum response with 10^{-9} M concentration was obtained, then the subsequent doses were added without washing the previous dose. This group served as control. Group 1 and dose response curve of serotonin was compared with that of fluoxetine to determine the magnitude of fluoxetine-mediated contraction, because in research studies Ach-mediated smooth muscle contraction is commonly taken as a standard (100%).

Group 2: Cumulative concentration-response curve of 5-HT (n=6)

Cumulative concentration-response curve of 5-HT was obtained using varying concentrations ranging from 10^{-9} to 10^{-6} M and the same procedure was repeated as described for acetylcholine.

Group 3: Cumulative concentration-response curve of

fluoxetine (n=6)

Cumulative dose response curve was constructed using varying concentrations of fluoxetine (10^{-9} - 10^{-6} M), and the same procedure is repeated as for Groups 1, and 2.

Statistical analysis

The results have been expressed as means \pm standard error of means (SEM). The arithmetic means of amplitudes of contractions and SEMs were calculated using SPSS version 17 (SPSS Inc.,

Chicago, IL, USA). In order to find the significance of the difference between two observations "Student's t-test" was used.

RESULTS

Acute effects of fluoxetine were studied on isolated ileal smooth muscles of rabbit by adding the successive doses of fluoxetine ranging from 10^{-9} to 10^{-6} M. Fluoxetine induced contraction of ileal smooth muscle was evident at a concentration of 10^{-9} M. However, a significant decrease of fluoxetine-induced contractions was observed at 10^{-8} M, 10^{-7} M and 10^{-6} M concentrations (Table 1). Ach mediated intestinal contraction is commonly taken as standard in experimental studies, so to evaluate the magnitude of fluoxetine-induced ileal contractility, acute effects of Ach and 5-HT on isolated ileal smooth muscle were also studied and were compared with constrictor response of fluoxetine (Table 1). Changes in ileal smooth muscle contractions were measured by taking the amplitudes of ileal smooth muscle contraction. Amplitudes of contraction with a maximum dose of Ach and 5-HT (10^{-6} M) were 24.8 ± 1.22 mm and 44 ± 0.527 mm respectively (Table 1). Hence, Ach and 5-HT significantly enhanced the myogenic ileal smooth muscle tone. To evaluate the extent of 5-HT-induced ileal contractility the percentage responses for Groups 1 and 2 were also calculated. Maximum constrictor response of 5-HT was 77% of maximal Ach response (Figure 1).

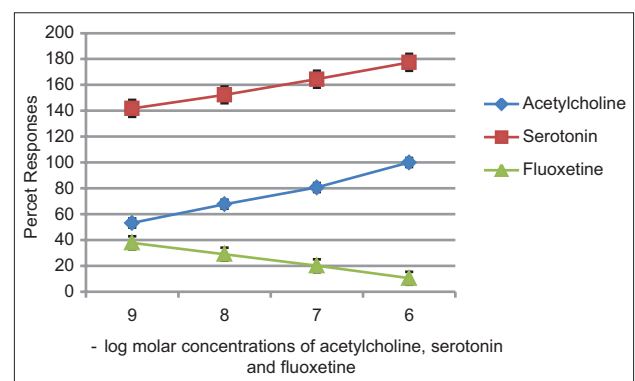


Figure 1: Comparison of increasing concentration of acetylcholine, serotonin and fluoxetine on isolated ileal smooth muscle.

Table 1: Amplitude of contractions and percent responses of acetylcholine, serotonin and fluoxetine.

Concentration (M)	Amplitude of contractions (Mean±SEM) mm Acetylcholine (n=6)	Percent response Acetylcholine	Amplitude of contractions (Mean±SEM) mm Serotonin (n=6)	Percent response Serotonin	Amplitude of contractions (Mean±SEM) mm Fluoxetine (n=6)	Percent response Fluoxetine
10 ⁻⁹	13.2±1	53.21	35.2±0.544	141.8	9.4±0.453	37.90
10 ⁻⁸	16.8±1.6	67.73	37.8±0.494	152.3	7.2±2.09	29.03
10 ⁻⁷	20±1.5	80.64	40.8±0.494	164.4	5±2.095	20.16
10 ⁻⁶	24.8±1.22	100	44±0.527	177.4	2.6±1.16	10.48
p-value	<0.05*					

*p-value significant, p value not significant. SEM: Standard error of means

This fluoxetine-induced ileal smooth muscle contraction was significantly reduced from 24.8±1.22 mm to 2.6±1.16 mm. The means of amplitudes of contractions with varying doses of fluoxetine, when compared between Groups 1 and 3 were found to be statistically significant (Table 1). Our data showed that maximum constrictor response of fluoxetine (Group 3) was reduced by 10% as compared with Ach group (Table 1). Fluoxetine concentration response curve was shifted to the right and downward indicating a profound inhibitory effect on ileal smooth muscle contractility (Figure 1).

DISCUSSION

The current study was undertaken to observe the acute effects of fluoxetine on ileal smooth muscle of rabbit and explored one possible mechanism that may underlies the acute contractile effect of fluoxetine on isolated ileal smooth muscle of rabbit. Ach and 5-HT increased the ileal smooth muscle contractility in a dose-dependent manner. The magnitude of fluoxetine-mediated ileal smooth muscle contractility was evaluated by comparing it with Ach-mediated ileal tissue contraction as Ach provoked ileal contractions is taken as a standard (control) for experimental studies.⁹

5-HT produced a dose dependent and sustained contractile response, which was 177% of Ach mediated response on ileal smooth muscles of rabbit. 5-HT receptor type 4 (5-HT₄) receptors are G-protein coupled receptors and are located on both cholinergic interneurons and motor neurons.¹⁰ The stimulation of 5-HT₄ receptors causes an increase in the Ach release which in turn increases the intestinal motor activity.¹¹

5-HT has diverse motor and sensory functions in the gastrointestinal tract through submucosal and myenteric neurons. 5-HT influences the gastrointestinal motility by acting directly through 5-HT₄ receptors on enterocytes and indirectly via 5-HT₃ receptors on mucosal nerves and vagal afferents.¹²

The effect of increasing concentration of fluoxetine on ileal smooth muscles of rabbit produced a decrease in contractile

response in our study. However, fluoxetine increases the motility of the gut due to its ability to inhibit serotonin transporter (SERT) as manifested by a decrease in orocecal transit time in past study.² This obvious contradiction may be due to the fact that the later study was a clinical trial conducted on human subjects, possibly the ability of fluoxetine to increase the concentration of 5-HT in the serotonergic synapse by blocking SERT is minimal in the isolated tissue. In addition to that fluoxetine may have a non-selective anticholinergic action as well which in the isolated tissue become more pronounced in the absence of enhanced serotonergic effect.³

Fluoxetine is liable to produce gastrointestinal side effects nausea, diarrhea, and other gastrointestinal symptoms. These adverse effects tend to occur early with treatment of these agents and situation improves after 1st week of therapy. This improvement may possibly be due to desensitization and down regulation of SERT in the gastrointestinal tract,¹³ or it may be due interaction with other receptors. Few studies do indicate the interaction of fluoxetine with these receptors but the magnitude of this interaction has not been explored.⁶ This magnitude of interaction with other receptors can be explored if we see the contractile effects of fluoxetine in the presence of muscarinic and anti-muscarinic drugs also, which will help us in further clarification of its gastrointestinal untoward effects.

CONCLUSION

Fluoxetine decrease the motility of isolated ileal intestinal smooth muscle and this decrease in motility are possibly due to the inability of fluoxetine in vitro to enhance the serotonergic transmission and also because of the interaction of these agents with some of the other receptors present in the intestinal smooth muscles.³

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethical Committee

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