Research Article

Fluoxetine causes decrease in intestinal motility

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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 neurotropic 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 rabbit in vitro. 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).

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.

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 ± 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

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**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.²

**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 ceacum 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% carbon dioxide.³ The cumulative dose response curve was made without 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 a research grade force displacement transducer of the oxygen tube in the tissue bath, and the other end 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.⁶ 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 10⁻⁶ 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 10⁻⁶ to 10⁻⁴ M and the same procedure was repeated as described for acetylcholine.

**Group 3: Cumulative concentration-response curve of**

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 ± 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 10⁻⁶ 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 contractility is commonly taken as standard in experimental studies, so to evaluate the magnitude of fluoxetine-induced ileal contractivity, 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.](image-url)
stimulation of 5-HT4 receptors causes an increase in the Ach smooth muscles of rabbit produced a decrease in contractile effects of fluoxetine on ileal smooth muscle of rabbit. Ach and 5-HT increased the ileal 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. The magnitude of this interaction has not been explored. The stimulation of 5-HT3 receptors causes an increase in the Ach release which in turn increases the intestinal motor activity. 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-HT3 receptors on enterocytes and indirectly via 5-HT3 receptors on mucosal nerves and vagal afferents.

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. 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.

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, 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.

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 underlie 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.

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. The stimulation of 5-HT4 receptors causes an increase in the Ach release which in turn increases the intestinal motor activity. 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.

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

| 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±0.494                                              | 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

| 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 |
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| 10^-9            | 13.2±1                                                      | 53.21                         | 35.2±0.494                                              | 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*                                                      |                                |                                                        |                           |                                                        |                                |

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REFERENCES

1. Ferrés-Coy A, Pilar-Cuellar F, Vidal R, Paz V, Masana M, Cortés R, et al. RNAi-mediated serotonin transporter suppression rapidly increases serotonergic neurotransmission and hippocampal neurogenesis. Transl Psychiatry. 2013;3:e211.
2. Camilleri M. Serotonergic modulation of visceral sensation: lower gut. Gut. 2002;51 Suppl 1:i81-6.
3. Coates MD, Johnson AC, Greenwood-Van Meerveld B, Mawe GM. Effects of serotonin transporter inhibition on gastrointestinal motility and colonic sensitivity in the mouse. Neurogastroenterol Motil. 2006;18(6):464-71.
4. Rand JB. Acetylcholine. WormBook. 2007;1-21.
5. Noor A, Najmi MH, Bukhtiar S. Effect of Montelukast on bradykinin-induced contraction of isolated tracheal smooth muscle of guinea pig. Indian J Pharmacol. 2011;43(4):445-9.
6. Jabeen Q, Aziz N, Afzal Z, Gilani HA. The spasmogenic and spasmolytic activities of lavandula stoechas are mediated through muscarinic receptor stimulation and calcium channel blockade. Int J Pharmacol. 2007;3(1):61-7.
7. Tuladhar BR, Costall B, Naylor RJ. Modulation of 5-HT4 receptor function in the rat isolated ileum by fluoxetine: the involvement of endogenous 5-hydroxytryptamine. Br J Pharmacol. 2002;136(1):150-6.
8. Tanko Y, Alladey O, Ahmad KM, Muhammad A, Musa KY. The effect of methanol leaves extract of Ficus Glumosa on gastrointestinal motility and castor oil induced diarrhoe in laboratory animals. Scholar Res Libr. 2012;2(3):360-7.
9. Chetty N, Irving HR, Coupar IM. Activation of 5-HT3 receptors in the rat and mouse intestinal tract: a comparative study. Br J Pharmacol. 2006;148(7):1012-21.
10. Spiller R. Serotonergic modulating drugs for functional gastrointestinal diseases. Br J Clin Pharmacol. 2002;54(1):11-20.
11. Pithadia AB, Jain SM. 5-Hydroxytryptamine receptor subtypes and their modulators with therapeutic potentials. J Clin Med Res. 2009;1(2):72-80.
12. Mujezinovic I, Cupic V, Samajlovic A, Muminovic M. Identification of serotonergic (5-H1A-Type) receptor in broiler small intestine by application of its serotonin and antagonist. Vet Glasnick. 2011;65(2):51-9.
13. Carr GV, Lucki I. The role of serotonin receptor subtypes in treating depression: a review of animal studies. Psychopharmacology (Berl). 2011;213(2-3):265-87.

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