e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(1); 491-495

Original Research Article

# Comparative Evaluation of Serotonergic and Cholinergic Modulation of Intestinal Motility in Isolated Rabbit Ileum: An Experimental Study

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**Received: 01-10-2025 / Revised: 15-11-2025 / Accepted: 21-12-2025**
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**Conflict of interest: Nil**

**Abstract**

Gastrointestinal motility is regulated by complex neurohormonal mechanisms involving multiple neurotransmitter systems, with serotonin and acetylcholine playing pivotal roles in modulating intestinal smooth muscle contractility. This experimental study aimed to evaluate and compare the effects of serotonergic and cholinergic agents on intestinal motility using isolated rabbit ileum preparations. The study was conducted using Dale's organ bath assembly with ileal segments from six healthy rabbits. Contractile responses including amplitude and frequency were recorded following exposure to acetylcholine chloride ($10^{-6}$M), serotonin hydrochloride ($10^{-6}$M), and fluoxetine ($1.4\times10^{-5}$M). Basal amplitude and frequency were 5.50±2.25 mm and 8.16±2.85 contractions/min respectively. Acetylcholine significantly increased amplitude (8.00±2.75 mm, $p<0.01$) and frequency (13.66±4.32/min, $p<0.01$) compared to baseline. Serotonin produced comparable excitatory effects with amplitude of 7.41±3.20 mm ($p<0.05$) and frequency of 17.16±5.19/min ($p<0.01$). Conversely, fluoxetine demonstrated inhibitory effects, reducing amplitude to 2.58±2.01 mm ($p<0.01$) and frequency to 4.33±2.94/min ($p<0.01$). The percentage increase in frequency was highest with serotonin (110.3%) compared to acetylcholine (67.4%), while fluoxetine produced 46.9% reduction from baseline. These findings demonstrate that while direct serotonergic stimulation enhances gut motility comparable to cholinergic activation, selective serotonin reuptake inhibition paradoxically decreases intestinal contractility, potentially through receptor desensitization mechanisms. The study provides experimental evidence supporting the differential roles of serotonergic pathways in gastrointestinal physiology and has implications for understanding drug-induced gastrointestinal effects.

**Keywords:** Gut motility, serotonin, acetylcholine, fluoxetine, rabbit ileum, organ bath, intestinal contractility.
**DOI:** 10.25258/ijcpr.18.1.80



## Introduction

The gastrointestinal tract represents the largest repository of serotonin (5-hydroxytryptamine, 5-HT) in the human body, with approximately 95% of total body serotonin located within the gut, primarily in enterochromaffin cells and enteric neurons [1]. This neurotransmitter plays a crucial role in regulating various gastrointestinal functions including motility, secretion, and visceral sensitivity through interaction with multiple receptor subtypes distributed throughout the enteric nervous system and smooth muscle layers [2].

Intestinal motility is orchestrated through the coordinated activity of the enteric nervous system, often referred to as the "second brain," which contains intrinsic neural circuits capable of autonomous function [3]. The primary excitatory neurotransmitter in this system is acetylcholine, which activates muscarinic receptors on smooth muscle cells to produce contraction. However, serotonin serves as an important modulatory neurotransmitter that can either enhance or inhibit motility depending on the receptor subtype activated and the specific intestinal region involved [4].

The $5\text{-HT}_3$ and $5\text{-HT}_4$ receptor subtypes have been identified as particularly important in gastrointestinal motility regulation. Activation of $5\text{-HT}_4$ receptors enhances peristaltic reflexes and accelerates gastrointestinal transit, while $5\text{-HT}_3$ receptors modulate visceral afferent signaling and influence nausea and vomiting reflexes [5]. This understanding has led to the development of prokinetic agents targeting serotonergic pathways for treatment of motility disorders.

Selective serotonin reuptake inhibitors (SSRIs), widely prescribed for depression and anxiety disorders, exert their therapeutic effects by blocking the serotonin transporter (SERT) and increasing synaptic serotonin availability [6]. However, given the abundance of serotonin in the gastrointestinal tract, SSRIs frequently produce gastrointestinal side effects including nausea, diarrhea, or constipation, the mechanisms of which remain incompletely understood [7].

Previous studies have demonstrated variable effects of SSRIs on gastrointestinal motility, with some reporting increased transit time while others document decreased contractility [8]. These discrepancies may reflect differences in experimental models, drug concentrations, or acute versus chronic exposure paradigms. The rabbit ileum preparation has been extensively validated as a reliable model for studying intestinal pharmacology due to its robust spontaneous rhythmic contractions and responsiveness to various pharmacological agents [9].

Despite extensive research on individual neurotransmitter systems, comparative studies evaluating the relative contributions of serotonergic and cholinergic mechanisms to intestinal motility in standardized experimental conditions remain limited. Furthermore, the acute effects of SSRI exposure on isolated intestinal tissue warrant investigation to better understand the immediate pharmacological consequences of serotonin reuptake inhibition.

The aim of this study was to comparatively evaluate the effects of acetylcholine, serotonin, and fluoxetine on the contractile amplitude and frequency of isolated rabbit ileum preparations, thereby elucidating the differential roles of cholinergic and serotonergic pathways in intestinal motility regulation.

## Materials and Methods

**Study Design and Setting:** This experimental in-vitro study was conducted in the Experimental Laboratory of the Department of Physiology, BRD Medical College, Gorakhpur, over a period of nine months. The study protocol was approved by the Institutional Animal Ethics Committee in accordance with guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Animals:** Six healthy adult rabbits of either sex, weighing between 2.0 and 4.5 kg, were procured from a registered breeder. Animals were housed in the institutional animal house under controlled environmental conditions (temperature 22±2°C, relative humidity 50-60%, 12-hour light-dark cycle) for one week prior to experimentation to allow acclimatization. Animals were provided standardized diet and water ad libitum during the acclimatization period.

**Chemicals and Drugs:** The following chemicals and drugs were used: Acetylcholine chloride ($10^{-6}$M), Serotonin (5-HT) Carnitine Sulfate (22µg/ml, $10^{-6}$M), Fluoxetine hydrochloride (4.3µg/ml, $1.4\times10^{-5}$M), and Amitriptyline hydrochloride (0.32µg/ml, $10^{-6}$M). All solutions were prepared fresh in distilled water immediately before experimentation.

**Tyrode's Solution Preparation:** Tyrode's solution was prepared with the following composition (g/L): NaCl (8.0), Glucose (1.0), KCl (0.20), $CaCl_2$ (0.20), $MgCl_2$ (0.10), $NaH_2PO_4$ (0.05), and $NaHCO_3$ (1.00). The solution was adjusted to pH 6.5 and maintained at 37±1°C throughout the experiment.

**Tissue Preparation:** Rabbits were fasted overnight with free access to water before sacrifice. Animals were anesthetized using diethyl ether inhalation. Following adequate anesthesia, a midline abdominal incision was made using surgical scissors to expose the intestines. The ileum was identified 5 cm proximal to the ileocecal junction, and segments of approximately 1.5 cm were excised. Tissue segments were immediately placed in a petri dish containing warm, oxygenated Tyrode's solution. Intestinal contents were gently flushed, and the solution was changed if contaminated.

**Organ Bath Assembly:** The Dale's organ bath assembly consisted of an electrically heated tank maintained at 37°C using a temperature thermostat. The bath was filled with Tyrode's solution and continuously aerated with 95% $O_2$ and 5% $CO_2$ mixture. Ileal segments were mounted vertically in the organ bath; the lower end was attached to a glass tube support via silk thread ligature, and the upper end was connected to an isometric force transducer under an initial tension of 2 g.

**Recording Procedure:** Tissue preparations were allowed to equilibrate for 30 minutes before recording commenced, with the bathing solution changed every 10 minutes during this period. Isometric contractions were amplified using a bridge amplifier and digitized via an analog/digital interface (Medicaid System Neuro Lab). Data were acquired on a personal computer and analyzed using Chart-5 software for Windows. Calibration for tension (0-10 g) was performed before and after each recording session.

**Experimental Protocol:** Following stabilization, spontaneous contractions were recorded for 15 minutes without intervention to establish baseline parameters. Subsequently, drugs were added individually to the organ bath, and contractile responses were recorded until maximum effect was achieved.

Between drug applications, the bath was drained and refilled with fresh Tyrode's solution, allowing adequate washout time (minimum 10 minutes) for tissue recovery to baseline activity. Amplitude (mm) and frequency (contractions per minute) were measured for each condition.

**Statistical Analysis:** Data were expressed as mean ± standard deviation (SD). Statistical comparisons between baseline and drug-treated conditions were performed using paired t-tests.

Comparisons among multiple drug groups were conducted using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test. A p-value less than 0.05 was considered statistically significant.

**Results**

**Baseline Contractile Parameters:** All six ileal preparations demonstrated stable spontaneous rhythmic contractions during the equilibration period. The mean basal amplitude was 5.50±2.25 mm with a range of 3-9 mm across preparations. The mean basal frequency was 8.16±2.85 contractions per minute, ranging from 4-12 contractions/min.

**Effect of Acetylcholine on Intestinal Motility:** Acetylcholine chloride ($10^{-6}$M) produced significant excitatory effects on ileal contractility. The mean amplitude increased from 5.50±2.25 mm to 8.00±2.75 mm, representing a 45.5% increase from baseline ($p<0.01$). Frequency increased from 8.16±2.85 to 13.66±4.32 contractions/min, a 67.4% enhancement ($p<0.01$). The response was rapid in onset, reaching maximum effect within 30-60 seconds of drug addiction.

**Table 1: Effect of Pharmacological Agents on Amplitude of Ileal Contractions**

| Experiment No. | Basal (mm) | Acetylcholine (mm) | Serotonin (mm) | Fluoxetine (mm) |
|---|---|---|---|---|
| 1 | 3 | 6 | 3.5 | 1 |
| 2 | 4 | 6 | 7 | 1.5 |
| 3 | 4 | 5 | 6 | 1 |
| 4 | 6 | 10 | 12 | 2 |
| 5 | 9 | 12 | 10 | 6 |
| 6 | 7 | 9 | 8 | 4 |
| **Mean±SD** | **5.50±2.25** | **8.00±2.75*** | **7.41±3.20*** | **2.58±2.01*** |
| % Change | — | +45.5% | +34.7% | -53.1% |

***p<0.05 vs baseline; **p<0.01 vs baseline**

**Effect of Serotonin on Intestinal Motility:** Serotonin ($10^{-6}$M) produced excitatory effects comparable to acetylcholine. Amplitude increased to 7.41±3.20 mm, representing a 34.7% increase from baseline ($p<0.05$). Notably, the effect on frequency was more pronounced than acetylcholine, with mean frequency increasing to 17.16±5.19 contractions/min, a 110.3% enhancement ($p<0.01$).

The onset of action was slightly slower than acetylcholine, with maximum effect achieved within 60-90 seconds.

**Table 2: Effect of Pharmacological Agents on Frequency of Ileal Contractions**

| Experiment No. | Basal (/min) | Acetylcholine (/min) | Serotonin (/min) | Fluoxetine (/min) |
|---|---|---|---|---|
| 1 | 9 | 10 | 13 | 3 |
| 2 | 12 | 20 | 26 | 9 |
| 3 | 8 | 13 | 15 | 5 |
| 4 | 10 | 16 | 20 | 6 |
| 5 | 6 | 15 | 17 | 2 |
| 6 | 4 | 18 | 12 | 1 |
| **Mean±SD** | **8.16±2.85** | **13.66±4.32*** | **17.16±5.19*** | **4.33±2.94*** |
| % Change | — | +67.4% | +110.3% | -46.9% |

***p<0.01 vs baseline**

**Effect of Fluoxetine on Intestinal Motility:** In contrast to direct neurotransmitter agonists, fluoxetine ($1.4\times10^{-5}$M) produced significant inhibitory effects on ileal motility. Amplitude decreased to 2.58±2.01 mm, representing a 53.1% reduction from baseline ($p<0.01$). Frequency was similarly suppressed to 4.33±2.94 contractions/min, a 46.9% reduction ($p<0.01$). The inhibitory effect developed gradually over 2-3 minutes and was sustained throughout the observation period.

**Table 3: Comparative Analysis of Drug Effects on Ileal Motility Parameters**

| Parameter | Basal | Acetylcholine | Serotonin | Fluoxetine | p-value (ANOVA) |
|---|---|---|---|---|---|
| Amplitude (mm) | 5.50±2.25 | 8.00±2.75 | 7.41±3.20 | 2.58±2.01 | <0.001 |
| Frequency (/min) | 8.16±2.85 | 13.66±4.32 | 17.16±5.19 | 4.33±2.94 | <0.001 |
| Amplitude change (%) | — | +45.5 | +34.7 | -53.1 | — |
| Frequency change (%) | — | +67.4 | +110.3 | -46.9 | — |

Post-hoc analysis revealed significant differences between fluoxetine and both acetylcholine ($p<0.001$) and serotonin ($p<0.001$) for both parameters. The difference between acetylcholine and serotonin effects was not statistically significant for amplitude ($p=0.872$) but showed a trend toward significance for frequency ($p=0.087$).

## Discussion

The present study demonstrates differential effects of cholinergic and serotonergic agents on isolated rabbit ileum contractility. Both acetylcholine and serotonin produced significant excitatory effects, while fluoxetine paradoxically inhibited intestinal motility. These findings contribute to understanding the complex neuropharmacological regulation of gastrointestinal function.

The excitatory effect of acetylcholine observed in this study is consistent with its established role as the primary excitatory neurotransmitter in the enteric nervous system. Acetylcholine activates muscarinic $M_3$ receptors on intestinal smooth muscle cells, leading to increased intracellular calcium and enhanced contractility [10]. The magnitude of response observed (45.5% increase in amplitude, 67.4% increase in frequency) aligns with previous reports using similar experimental preparations.

The comparable excitatory effects of serotonin support its recognized prokinetic role in gastrointestinal physiology. Serotonin released from enterochromaffin cells activates intrinsic primary afferent neurons expressing 5-$HT_4$ receptors, initiating peristaltic reflexes [11]. The particularly pronounced effect on frequency (110.3% increase) suggests that serotonin may preferentially modulate the pacemaker mechanisms governing intestinal rhythmicity rather than the contractile apparatus itself. The inhibitory effect of fluoxetine represents a notable finding that warrants careful interpretation. While SSRIs increase synaptic serotonin availability through transporter blockade, acute exposure to high concentrations may produce paradoxical effects through various mechanisms [12]. One possibility involves rapid desensitization of 5-HT receptors due to excessive serotonin accumulation at the synapse. Additionally, fluoxetine has been reported to possess direct inhibitory effects on L-type calcium channels independent of serotonin reuptake inhibition [13].

Studies examining the acute effects of SSRIs on gastrointestinal smooth muscle have reported variable findings depending on the specific agent, concentration, and tissue preparation used [14]. The concentration of fluoxetine employed in this study ($1.4\times10^{-5}$M) represents a supratherapeutic level that may not directly correlate with clinical exposure. However, high tissue concentrations may occur locally in the intestinal wall following oral administration, potentially explaining some acute gastrointestinal side effects.

The contrast between direct serotonin application (excitatory) and serotonin reuptake inhibition (inhibitory) highlights the complexity of serotonergic signaling in the gut. Under physiological conditions, serotonin is rapidly cleared from the synaptic cleft by SERT, allowing precise temporal control of receptor activation [15]. Blocking this reuptake mechanism may result in sustained receptor activation leading to desensitization, effectively producing a functional antagonism despite increased serotonin levels.

Clinical observations support the relevance of these findings. Patients initiating SSRI therapy frequently report gastrointestinal disturbances including both diarrhea and constipation, suggesting complex and potentially region-specific effects on gut motility [16]. The initial inhibitory phase observed in acute exposure may transition to enhanced motility with chronic treatment as compensatory mechanisms develop.

Limitations of this study include the small sample size, use of a single concentration for each drug, and the in-vitro nature of the preparation which may not fully replicate the complex in-vivo environment. Additionally, the isolated ileum preparation excludes extrinsic neural influences that normally modulate intestinal function.

## Conclusion

This experimental study demonstrates that both acetylcholine and serotonin produce significant excitatory effects on isolated rabbit ileum contractility, with serotonin showing particularly pronounced effects on contraction frequency. Conversely, fluoxetine produces acute inhibitory effects on intestinal motility despite its mechanism of enhancing serotonergic transmission. These findings highlight the complex and concentration-dependent nature of serotonergic modulation in the gastrointestinal tract. The differential effects of direct serotonin receptor activation versus serotonin reuptake inhibition have important implications for

understanding drug-induced gastrointestinal effects and developing targeted therapeutic strategies for motility disorders. Further studies examining dose-response relationships and chronic exposure effects are warranted to fully elucidate these mechanisms.

## References


1. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology. 2007;132(1):397-414. doi: 10.1053/j.gastro.2006.11.002. PMID: 17241888.
2. Mawe GM, Hoffman JM. Serotonin signalling in the gut--functions, dysfunctions and therapeutic targets. Nat Rev Gastroenterol Hepatol. 2013;10(8):473-86. doi: 10.1038/nrgastro.2013.105. PMID: 23797870.
3. Furness JB. The enteric nervous system and neuro gastroenterology. Nat Rev Gastroenterol Hepatol. 2012;9(5):286-94. doi: 10.1038/nrgastro.2012.32. PMID: 22392290.
4. Spencer NJ, Hu H. Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility. Nat Rev Gastroenter ol Hepatol. 2020;17(6):338-351. doi: 10.1038/s41575-020-0271-2. PMID: 32152479.
5. Camilleri M. Serotonin in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes. 2009; 16(1):53-9. doi: 10.1097/MED.0b013e32831e9c8e. PMID: 19115522.
6. Stahl SM. Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. J Affect Disord. 1998; 51(3):215-35. doi: 10.1016/s0165-0327(98)00221-3. PMID: 10333979.
7. Weinrieb RM, Auriacombe M, Lynch KG, Lewis JD. Selective serotonin re-uptake inhibitors and the risk of bleeding. Expert Opin Drug Saf. 2005;4(2):337-44. doi: 10.1517/14740338.4.2.337. PMID: 15794724.
8. Gorard DA, Libby GW, Farthing MJ. Influence of antidepressants on whole gut and orocaecal transit times in health and irritable bowel syndrome. Aliment Pharmacol Ther. 1994; 8(2):159-66. doi: 10.1111/j.1365-2036.1994.tb00273. x. PMID: 8038347.
9. Bolton TB, Prestwich SA, Zholos AV, Gordienko DV. Excitation-contraction coupling in gastrointestinal and other smooth muscles. Annu Rev Physiol. 1999;61:85-115. doi: 10.1146/annurev.physiol.61.1.85. PMID: 10099683.
10. Unno T, Matsuyama H, Sakamoto T, Uchiyama M, Izumi Y, Okamoto H, Yamada M, Wess J, Komori S. M(2) and M(3) muscarinic receptor-mediated contractions in longitudinal smooth muscle of the ileum studied with receptor knockout mice. Br J Pharmacol. 2005;146(1):98-108. doi: 10.1038/sj.bjp.0706300. PMID: 15965503.
11. Grider JR, Piland BE, Gulick MA, Qiao LY. Brain-derived neurotrophic factor augments peristalsis by augmenting 5-HT and calcitonin gene-related peptide release. Gastroenterology. 2006;130(3):771-80. doi: 10.1053/j.gastro.2005.12.026. PMID: 16530518.
12. 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. doi: 10.1111/j.1365-2982.2006.00792. x. PMID: 16700726.
13. Bhutada PS, Mundhada YR, Bansod KU, Dixit PV, Umathe SN, Mundhada DR. Inhibitory influence of fluoxetine on contractility of isolated smooth muscle preparations. Indian J Physiol Pharmacol. 2009;53(4):361-8. PMID: 20509328.
14. Chial HJ, Camilleri M, Burton D, Thomforde G, Olden KW, Stephens D. Selective effects of serotonergic psychoactive agents on gastrointestinal functions in health. Am J Physiol Gastrointest Liver Physiol. 2003;284(1): G130-7. doi: 10.1152/ajpgi.00266.2002. PMID: 12488239.
15. Chen JJ, Li Z, Pan H, Murphy DL, Tamir H, Bhide PG, Bhide K, Bhide MD, Bhide GM. Maintenance of serotonin in the intestinal mucosa and ganglia of mice that lack the high-affinity serotonin transporter. Gastroenterology. 2001;121(2):329-39. doi: 10.1053/gast.2001.26220. PMID: 11487543.
16. Masand PS, Gupta S. Selective serotonin-reuptake inhibitors: an update. Harv Rev Psychiatry. 1999;7(2):69-84. doi: 10.3109/hrp.7.2.69. PMID: 10471245.