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Characteristics of 5-Hydroxytryptamine Receptors Involved in Contraction of Feline Ileal Longitudinal Smooth Muscle

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A number of studies have demonstrated that 5-hydroxytryptamine (5-HT) can induce muscle contraction or relaxation response and enhance secretion in the gastrointestinal tract via a multiplicity of 5-HT receptor subtypes. In the present study, we investigated the pharmacological characterization of the 5-HT-induced contractile response in longitudinal smooth muscle isolated from the feline ileum. Addition of 5-HT into muscle chambers enhanced the basal tone and spontaneous activity in a concentration-dependent manner. The neurotoxin tetrodotoxin did not alter the 5-HT-induced contraction of the longitudinal muscles. Neither atropine nor guanethidine affected the contraction. The 5-HT agonists, 5-methylserotonin hydrochloride and mosapride, also evoked concentration-dependent contractions. The 5-HT-induced contraction was enhanced by the $5HT_2$ receptor antagonist ketanserin and the 5-HT_3 receptor antagonist ondansetron but was inhibited by the 5-HT_1 receptor antagonist methysergide and 5-HT_4 receptor antagonist GR113808. These results indicate that 5-HT_1 and 5-HT_4 receptors may mediate the contraction of the 5-HT-induced response and 5-HT_2 and 5-HT_3 receptors may mediate 5-HT-induced relaxation in feline ileal longitudinal smooth muscles.

Key Words: Ileum, Longitudinal smooth muscle, 5-HT, Contraction, Relaxation

INTRODUCTION

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter that is a key mediator in the physiology of mood, vascular function and gastrointestinal (GI) motility. 5-HT is known to be distributed in blood platelets, the GI tract and the central nervous system (CNS) of animals and humans [1-5]. Approximately 80% of the human body's total 5-HT is located in the enterochromaffin cells in the gut, where it is reported to be involved in intestinal movements via several 5-HT receptor subtypes [3,6]. Based on biochemical and pharmacological criteria, 5-HT receptors are classified into seven main receptor subtypes, five of which are present on enteric neurons, enterochromaffin cells and GI smooth muscles, namely 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 [7,8]. With the exception of the 5-HT_3 receptor, a ligand-gated ion channel, all 5-HT receptors are G protein-coupled receptors that activate an intracellular second messenger cascades to produce either excitatory or inhibitory responses [8].

A number of studies have characterized 5-HT-induced muscle contraction or relaxation responses and enhanced secretion in the GI tract via a multiplicity of 5-HT receptor subtypes [5,9-13]. For example, in ferrets, 5-HT_1 and 5-HT_3 receptors mediate contraction, whereas in piglets, 5-HT_1 receptors mediate the contraction in the ileum. In addition, 5-HT_2 receptors mediate contraction and 5-HT_4 receptors mediate relaxation in the rat stomach [11]. 5-HT_1 and 5-HT_2 receptors mediate contractions and 5-HT_4 receptors mediate the relaxation in the ileum [5,12]. In chickens, 5-HT_1- and 5-HT_2-like receptors mediate the contractions in the ileum [11]. In *Suncus murinus*, 5-HT_2 and 5-HT_3 receptors mediate contraction in the ileum [9], while 5-HT_3 receptor mediates the contraction in mouse ileum [13]. 5-HT_4-like receptors mediate the relaxation of human colon [14]. In such studies, guinea-pig, rat, mouse, ferret, chicken and human have been widely used [5,9-14]. However, the effect of 5-HT on the regulation of intestinal contraction or which 5-HT receptor is involved in the 5-HT-elicited response has not been established in isolated feline intestine.

Intestinal smooth muscles display rhythmic spontaneous activity due to the role of interstitial cells of Cajal (ICC) as pacemaker cells in the GI tract [15-18]. Spontaneous contractions of the longitudinal muscle in the ileum and colon play an important role in GI motility, including peristalsis, and are a useful tool to investigate the regulation of motor activity. The spontaneous contractions are also termed giant contractions (GCs). It has been reported that the am-

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; GI, gastrointestinal; CNS, central nervous system; ICC, interstitial cells of Cajal; GCs, giant contractions; TXT, tetrodotoxin; 5-MOT, 5-methoxytryptamine; DMSO, dimethyl sulfoxide; IBS, irritable bowel syndrome.

plitude of GCs of circular muscle strips are significantly increased by tetrodotoxin (TTX), but not affected by antagonists of 5-HT receptors in rat colon [19]. On the other hand, the migrating motor complexes in mouse colon have been reported to be inhibited by alosetron, a 5-HT_3 receptor antagonist, in vitro [20]. Moreover, Ca^{2+} influx plays a critical role in the spontaneous contraction of feline ileum with ICC pacing [18]. The aim of the present study is to clarify the pharmacological characterization of 5-HT-induced contractile response using selective agonist and antagonists of 5-HT receptor subtypes, and to investigate the potential effect of 5-HT on the basal spontaneous contractile activity in feline ileal longitudinal smooth muscles.

METHODS

Drugs and chemicals

5-hydroxytryptamine hydrochloride (Sigma), 5-methylserotonin hydrochloride (5-MOT, Sigma), mosapride citrate dehydrate (Sigma), methysergide maleate salt (Sigma), ketanserin (+)-tartrate salt (Sigma), ondansetron hydrochloride dihydrate (Sigma), atropine sulfate (Merck) and tetrodotoxin citrate (Tocris Cookson, Ltd.) were each dissolved in distilled water. GR113808 (1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate: Sigma) was dissolved in dimethyl sulfoxide (DMSO). The drug solutions were prepared on the day of the experiment and spontaneous contraction or resting tone was unaffected by the vehicle at volumes needed for dilution.

Preparation of tissue

All experimental procedures were performed in the accordance with the guidelines of the Institutional Animal Care and Use Committee of Chung-Ang University, Seoul, South Korea. Adult cats of either sex weighing between 3.0～4.0 kg were used in this study. Cats were anesthetized with ketamine (50 mg/ml/kg) and killed by an overdose of 25% urethane (Aldrich, St. Louis, MO, USA). The abdomen was opened with a midline incision and cleaned of fat tissue. The ileum was excised beginning at 7～10 cm proximal to the ileo-cecal junction and then immediately placed into Krebs solution of the following composition (mM): NaCl 118.6, $NaHCO_3$ 24, KH_2PO_4 1.2, KCl 4.8, $CaCl_2$ 2.5, glucose 11 and $MgSO_4$ 1.2 (pH 7.45), gassed with 95% O_2 and 5% CO_2 and cooled (4°C) before dissection. The ileal segments were opened, and the mucosal and submucosal layers were removed by blunt scissor dissection and discarded. The muscle tissue was cut into 4～5 strips with dimensions of approximately 2 mm in width and 10 mm in length parallel to the direction of the longitudinal axes.

Measurements of in vitro muscle spontaneous contraction

Muscle spontaneous contraction was measured by previously described method [18]. Briefly, The muscle strips were mounted in separated 1-ml muscle chambers and maintained at 37±0.5°C in Krebs solution (pH 7.45) and gassed continuously with a mixture of 95% O_2 and 5% CO_2 as follows: one end of each strip was fixed to the bottom of the muscle chamber and the other end was attached to an isometric muscle transducer (Polygraph, Grass Co., USA) for continuous monitoring of isometric tension. Changes in isometric force were recorded on a direct writing-ink pen polygraph (Grass model 79, Grass Instruments Co., Quincy, MA, USA). The muscle strips were initially stretched with 1 g of tension to bring them near conditions of optimal force development. They were equilibrated for 1 h with two washouts every 30 min while being perfused continuously with oxygenated Krebs solution. During this time, the tension of the ileal longitudinal smooth muscle strips decreased rapidly and reached a stable baseline tension of less than 0.5 g with initiation of spontaneous activity.

Experimental protocol

Concentration-response curves to 5-HT and other agonists were established by increasing the concentration of the agonists added to the organ bath with a 5-min contact time. To evaluate the effect of antagonists, the tissue strips were exposed to the antagonists 30 min before and during administration of agonists. The applied concentration of each antagonist was determined based on our preliminary trials or those reported in the literature. The responses to 5-HT in the absence and presence of the 5-HT antagonists methysergide, ketanserin, ondansetron and GR113808 were determined. The effect of 5-HT-induced contraction in the absence and presence of atropine, guanethidine and TTX was also tested. Muscle strips were equilibrated for 30 min after 5 washes with Krebs solution between each set of experiments.

Statistical analysis

The amplitude of the 5-HT-induced contraction was expressed as mean % of control of cycles per min before and after drug treatment. The frequencies of contractions in each experiment were measured and expressed as contraction cycles per min. Data are expressed as means±S.E., with n representing the number of animals used. Statistical significance was determined using a two-tailed Student's t-test for paired observations or one-way analysis of variance where appropriate. A difference was considered significant at $p < 0.05$. Traces are representative of at least three experiments on three or more muscle preparations. Doses of all drugs are reported in molar concentrations and refer to their final bath concentration.

RESULTS

Effects of atropine and guanethidine on 5-HT-induced response

Strips from the isolated longitudinal ileum displayed spontaneous phasic activity. Muscle response to 5-HT produced an increase of basal tone (hereafter named 5-HT-induced contraction) and spontaneous activity in a concentration-dependent manner (Fig. 1). To test whether cholinergic or adrenergic neural effect was implicated in 5-HT-induced contraction, muscle strips were incubated with atropine (central and peripheral muscarinic cholinergic receptor antagonist, 10^{-6} and 10^{-7} M) or guanethidine (adrenergic antagonist, 10^{-6} M and 10^{-7} M) for 10 min before and during exposure to 5-HT [21]. Atropine or guanethidine had no significant effect on 5-HT-induced increases

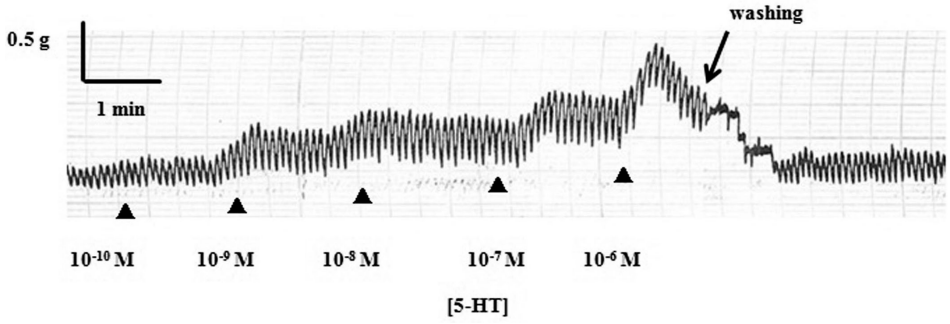

Fig. 1. Representative tracing showing the effect of 5-HT on the spontaneous contraction of feline longitudinal smooth muscle. 5-HT was added cumulatively (10^{-10}~10^{-6} M) with 5-min contact time for each concentration.

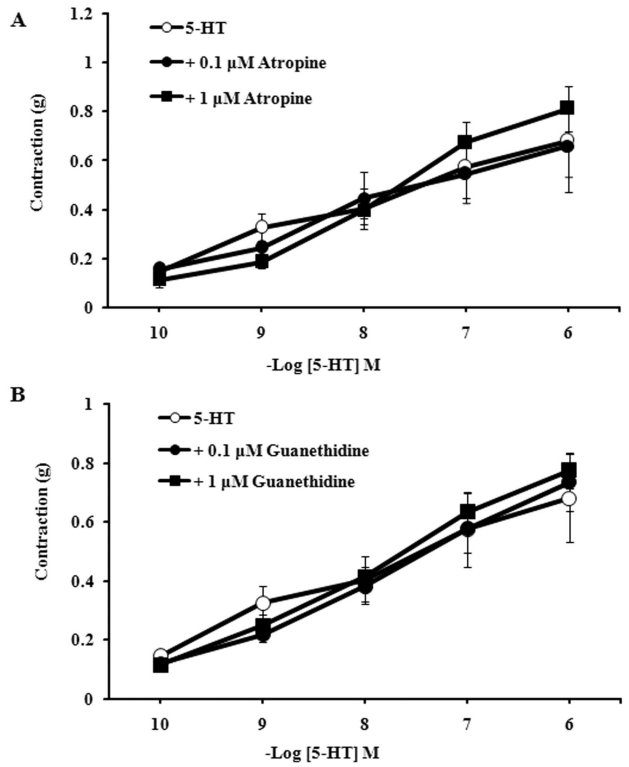

Fig. 2. Effect of atropine or guanethidine on 5-HT-induced contraction. Concentration-response curves to 5-HT in the absence (○) and presence of 0.1 μM atropine (●), (A) or 1 μM guanethidine (■), (B). Preparations were incubated with atropine or guanethidine for 10 min before and during 5-HT treatment. Each point represents the mean±S.E.M. (n=6).

in muscle basal tone (Fig. 2). These results suggest that cholinergic or adrenergic innervations were not involved in 5-HT-induced contraction of feline ileal longitudinal muscle.

Effect of TTX on 5-HT-induced contraction

In order to further test for possible neural control in the 5-HT-induced contraction, TTX (nerve electrical propagation blocker, 10^{-5} and 10^{-6} M) was incubated with the muscle strips for 10 min before and during 5-HT treatment. The 5-HT-induced contraction was unaffected by pretreatment with TTX (Fig. 3).

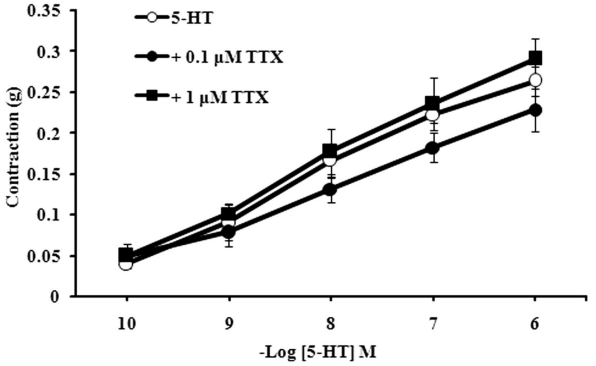

Fig. 3. Effect of TTX on 5-HT-induced contraction. Concentration-response curves to 5-HT in the absence (○) and presence of 0.1 μM (●) and 1 μM (■) TTX. Preparations were incubated with TTX for 10 min before and during 5-HT treatment. Each point represents the mean±S.E.M. (n=5).

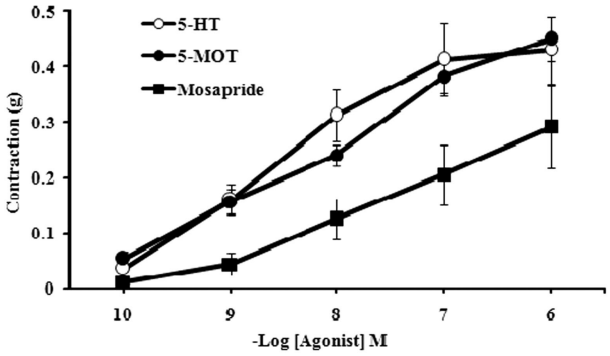

Fig. 4. Concentration-response curves for 5-HT- (○, n=11), 5-MOT- (●, n=8) and mosapride- (■, n=10) induced contraction. Each point represents the mean±S.E.M.

Effect of 5-HT and other 5-HT receptor agonists on basal muscle tone

The cumulative addition of 5-HT (10^{-10}~10^{-6} M) significantly evoked concentration-dependent increases in the basal tone of all segments. 5-MOT (a nonselective 5-HT receptor agonist, 10^{-10}~10^{-6} M), and mosapride (a selective 5-HT_4 receptor agonist, 10^{-10}~10^{-6} M) also evoked concentration-dependent contractions (Fig. 4).

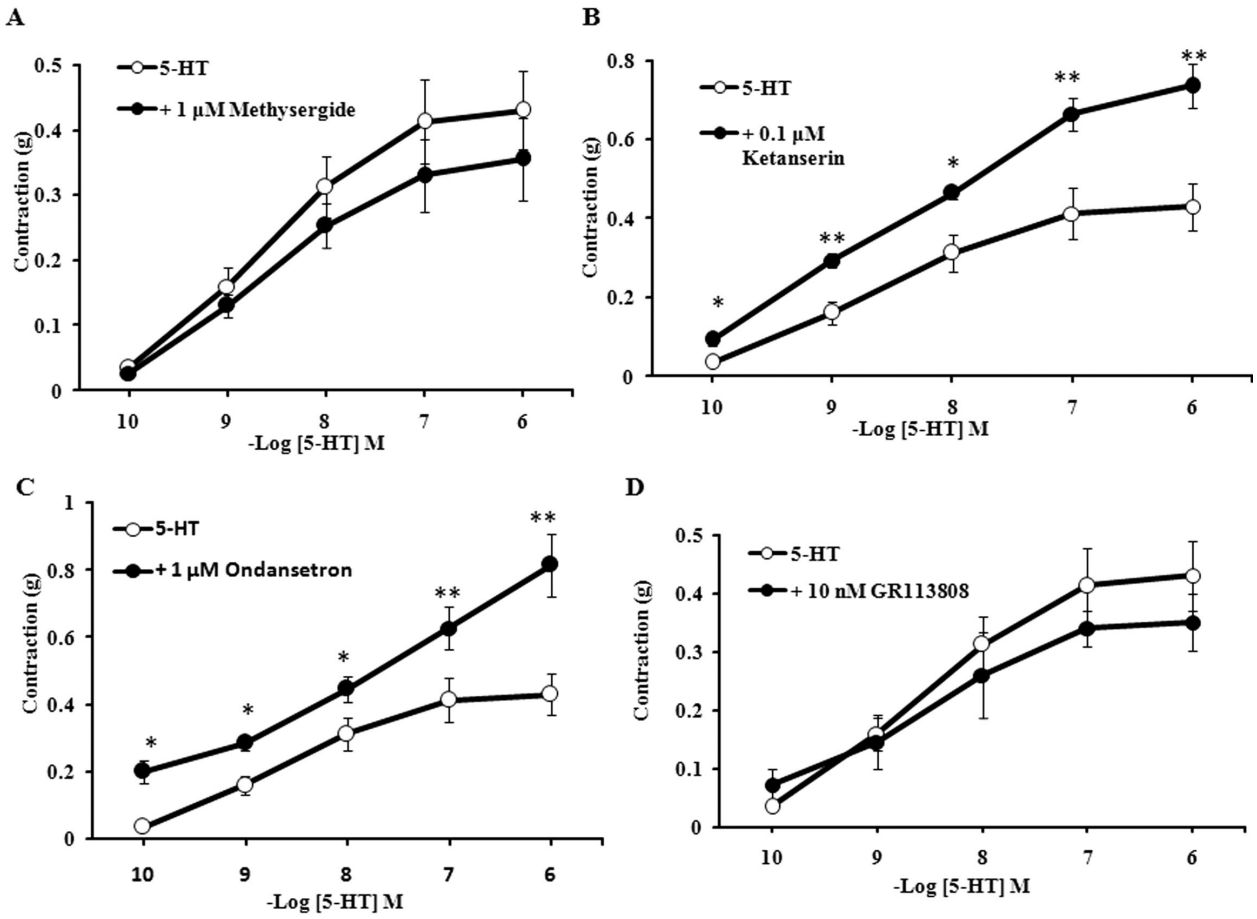

Fig. 5. Inhibitory effect of 5-HT receptor antagonists on 5-HT-induced contraction. The preparation was incubated with (A) methysergide (1 μM, n=5), (B) ketanserin (0.1 μM, n=8), (C) ondansetron (1 μM, n=8) or (D) GR113808 (10 nM, n=5) for 30 min before and during 5-HT treatment. Absence (○) or presence (●) of antagonist. Each point represents the mean±S.E.M. *$p<0.05$ and **$p<0.01$ compared to the same concentration of 5-HT-induced contraction alone.

Effects of 5-HT receptor antagonists on 5-HT-induced contraction

To test whether 5-HT_1 or 5-HT_2 receptors were involved in the 5-HT-induced contraction, muscle strips were pretreated with methysergide (a nonselective 5-HT_1 and 5-HT_2 receptor antagonist, 10^{-6} M) or ketanserin (a selective 5-HT_2 receptor antagonist, 10^{-7} M) for 30 min before addition of 5-HT. Methysergide did not produce any significant change in 5-HT-induced contraction (Fig. 5A). Pretreatment with ketanserin, on the other hand, significantly enhanced contraction of the ileum strips (Fig. 5B). To further test whether 5-HT_3 or 5-HT_4 receptors were involved in 5-HT-induced contraction, muscle strips were pretreated with ondansetron (a selective 5-HT_3 receptor antagonist, 10^{-6} M) or GR113808 (a selective 5-HT_4 receptor antagonist, 10^{-8} M) for 30 min before addition of 5-HT. Ondansetron significantly enhanced 5-HT-induced contraction (Fig. 5C), whereas GR113808 had no significant effect (Fig. 5D). These results suggest that the 5-HT_2 and 5-HT_3 receptors have a relaxation effect in the feline ileum, while the 5-HT_1 and 5-HT_4 receptors are involved in the contraction of feline ileum (Fig. 6).

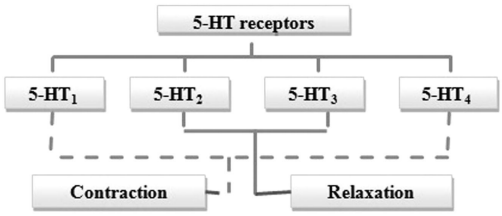

Fig. 6. Major roles of 5-HT receptors in isolated feline ileum longitudinal smooth muscle. 5-HT can stimulate 5-HT_1 or 5-HT_4 receptors resulting in contraction of smooth muscle. 5-HT can also stimulate 5-HT_2 or 5-HT_3 receptors resulting in relaxation of smooth muscle.

DISCUSSION

The effect of 5-HT in the mammalian cardiovascular system has been well elucidated. The tachycardia induced by 5-HT is mediated by the 5-HT_1 receptor in cat, the 5-HT_2 receptor in rat and dog, the 5-HT_3 receptor in rabbit and guinea pig, and the 5-HT_4 receptor in pigs and human [22-24]. Exogenous 5-HT affects not only the cardiovascular

system but also the gastrointestinal system in most animals and humans. Although several studies have examined the effect of 5-HT on intestinal motility in guinea pigs and rats [5,12], its effect in feline is not known.

The present study showed that cumulative addition of 5-HT dose-dependently increased the basal tone in the ileal longitudinal muscle of felines. Not only 5-HT, but also 5-MOT and mosapride concentration-dependently induced an increase in basal tone. Cholinergic or adrenergic innervations were not implicated in 5-HT-induced contraction. Moreover, contraction induced by 5-HT was not blocked by TTX, indicating that 5-HT-induced contraction did not involve enteric nerves. Based on these results, contraction in response to exogenous 5-HT was likely mediated by 5-HT receptors located directly on the longitudinal ileal smooth muscle in feline.

In our study, methysergide and GR113808 slightly inhibited 5-HT-induced contraction, but only at high concentration, and these effects were not statistically significant. As shown in Fig. 4, mosapride induced contraction. These results imply that 5-HT-induced contraction of the longitudinal ileal smooth muscle of feline was mediated, in part, through 5-HT_1 and 5-HT_4 receptors. Otherwise, it is possible that another 5-HT receptor subtype may highly participate in the contraction, resulting in an overwhelming contractile effect mediated by either 5-HT_1 or 5-HT_4 receptor. There is a difference in the mechanism mediating 5-HT-induced contraction between species. In contrast with our results, there was involvement of 5-HT_3 receptors in 5-HT-mediated contraction in mouse ileum, as well as involvement of 5-HT_1 and 5-HT_2 receptors in rat ileum [5,13]. Tuladhar et al. (2000) reported the involvement of 5-HT_1 and 5-HT_2 receptor in the relaxant response of mouse ileum. However, in our study, 5-HT-induced contraction was significantly enhanced by ketanserin and ondansetron, indicating that 5-HT_2 and 5-HT_3 receptors have a relaxing effect in isolated feline ileum longitudinal smooth muscles.

5-HT is an important signaling molecule in the gut enterocytes, smooth muscles and enteric neurons [25]. Exogenous 5-HT application evokes many responses, making it difficult to determine which is physiologically relevant since a variety of receptor subtypes are present on several classes of myenteric neurons, smooth muscle cells and epithelial cells. 5-HT initiates responses as diverse as nausea, vomiting, intestinal secretion and peristalsis, in addition to playing a role in bowel physiology as an enteric neurotransmitter [26]. There are many studies on to the role of 5-HT signaling in the pathophysiology of irritable bowel syndrome (IBS) [27-31]. It is well-known that IBS is a complex disorder associated with altered GI motility, secretion and sensation. 5-HT directly and indirectly affects intestinal motor and secretory function as well as abnormalities leading to either constipation or diarrhea in IBS. Therefore, therapeutic agents inducing alterations of serotonin signaling may provide new effective treatments for patients with IBS [31]. To do that, information about the characteristics of diverse 5-HT receptor family members present in the intestinal muscle is needed. Many observations on the 5-HT receptor subtypes have been accumulated: 5-HT_{1A} receptors mediate nonadrenergic inhibitory synaptic potentials in the noncholinergic secretomotor neurons [32]. 5-HT can induce porcine proximal stomach contraction via smooth muscle 5-HT_{2A} receptors [33]. Acetylcholine release induced by a neurokinin NK_3 receptor agonist is inhibited by stimulation of a 5-HT receptor, possibly of the 5-HT_{2B} or 5-HT_{2C} subtype [34]. Although our study did not detail the 5-HT receptor subtypes present in the ileal muscle, it revealed the different characteristics of the receptor subtypes in the intestinal smooth muscle. Therefore, development of drugs that target specific 5-HT receptor subtype subtypes may help to regulate disorders of intestinal motility.

In conclusion, we showed that 5-HT induced contractile responses through multiple 5-HT receptor subtypes located directly on the longitudinal muscle of isolated feline ileum. 5-HT_1 and 5-HT_4 receptors may mediate contraction in the 5-HT-induced response, whereas 5-HT_2 and 5-HT_3 receptors may mediate relaxation in the 5-HT-induced response. The diversity of expressed 5-HT receptor subtypes, in addition to the contractile responses to and affinities for 5-HT between species, might lead to the different results in response to 5-HT.

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