Fluoxetine Affects Intestinal Motility via 5-HT$_3$ and Muscarinic Receptors in *ex vivo* Mouse Model

(Kesan Fluoxetin terhadap Motiliti Usus melalui Reseptor 5-HT3 dan Reseptor Bermuskarina pada Model Tikus *ex vivo*)

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ABSTRACT

Fluoxetine, a selective serotonin reuptake inhibitor anti-depressant, causes undesirable side effects, including diarrhea and constipation. This research investigated the direct effects of fluoxetine at 0.001, 0.01, 0.1, 1, 10, and 100 μM on duodenal and proximal colonic tissue contractions. The investigation aimed to determine related mechanisms using an isolated mouse intestine model. Our study showed that fluoxetine at 0.001 μM increased the amplitude of contraction in colonic tissue but decreased the amplitude in duodenal tissue. The direct application of higher concentrations of fluoxetine (1, 10, and 100 μM) reduced the amplitude of contractions in proximal colonic tissue. Moreover, we found that the stimulatory effect of 0.001 μM fluoxetine on the tone of contractions could be prevented by pre-incubating the tissue in ondansetron and atropine. Our findings suggest that the inhibition of the effect of fluoxetine was mainly mediated via 5-HT$_3$ receptors and muscarinic signaling. These findings might explain the conflicting gastrointestinal symptoms caused by fluoxetine.

Keywords: Intestinal contraction; selective serotonin reuptake inhibitor; 5-hydroxytryptamine

ABSTRAK

Fluoxetin ialah perencat pengambilan anti-depresan serotonin yang memilih, menyebabkan kesan sampingan yang tidak diingini, termasuk cirit-birit dan sembelit. Penyelidikan ini mengkaji kesan langsung fluoxetin pada 0.001, 0.01, 0.1, 1, 10 dan 100 μM pada pengecutan tisu kolon duodenum dan proksimal. Penyelidikan bertujuan untuk menentukan mekanisme yang berkaitan dengan menggunakan model usus tikus yang terpencil. Kajian menunjukkan bahawa fluoxetin pada 0.001 μM meningkatkan amplitud penguncupan pada tisu kolon tetapi menurunkan amplitud pada tisu duodenum. Aplikasi langsung kepekatan fluoxetin yang lebih tinggi (1, 10 dan 100 μM) mengurangkan amplitud pengecutan pada tisu kolon proksimal. Selain itu, didapati bahawa kesan perangsang 0.001 μM fluoxetin pada nada pengecutan dapat dicegah dengan pra-inkubasi tisu dalam ondansetron dan atropin. Penemuan kami menunjukkan bahawa penghambatan kesan fluoxetin terutamanya dimediasi melalui reseptor 5-HT3 dan isyarat bermuskarina. Penemuan ini dapat menjelaskan gejala gastrointestin yang bertentangan yang disebabkan oleh fluoxetin.

Kata kunci: Pengecutan usus; perencat pengambilan serotonin selektif; 5-hidroksitriptamina

INTRODUCTION

Depression is a mental disorder that was recently reported to affect more than 264 million people worldwide (James et al. 2018) and has been linked to low levels of serotonin 5-hydroxytryptamine (5-HT) in the central nervous system (Colle et al. 2020). Patients suffering from depression are frequently treated with selective serotonin reuptake inhibitors (SSRIs), including fluoxetine. Generally, treatment with fluoxetine is effective at an initial dose of 20 mg/day. The recommended dosage range is 20 to 60 mg/day (Bastos et al. 2013). However, SSRIs cause undesirable side effects which can include insomnia, impaired sexual function, and gastrointestinal symptoms such as nausea, vomiting, and diarrhea (Gelenberg et al. 2010). 5-HT, the putative regulator of depressive disorders, is mainly produced in enterochromaffin cells and intrinsic enteric neurons of the gastrointestinal tract. 5-HT levels in the gastrointestinal tract are regulated via the serotonin transporter (SERT) in serotonergic neurons and intestinal epithelial cells (De Ponti 2004). However, the reuptake inhibition of 5-HT by fluoxetine is principally achieved by blocking the action of SERT, which increases extracellular concentrations of 5-HT (Kannen et al. 2011). Side effects of SSRIs on the gastrointestinal tract may be

due to increased availability of 5-HT that activates 5-HT$_3$ receptors, which can control gut motility by increasing transmitter release from enteric neurons. This activity accelerates gut transit, increases fluid secretions, and modulates visceral sensitivity (De Ponti 2004) and was linked to the side effects of fluoxetine (Costescu et al. 2019). Curiously, fluoxetine users may occasionally experience diarrhea alternated with constipation. These conflicting symptoms were associated with the plasticity of serotonergic mechanisms in the enteric nervous system of the gastrointestinal tract (Gershon & Tack 2007). However, the mechanisms related to these intestinal symptoms are still unclear.

Therefore, this study aimed to examine the direct effects of various concentrations of fluoxetine on duodenal and colonic motility. Although a previous study reported that atropine could competitively antagonize the action of the 5-HT$_3$ receptor (Lochner & Thompson 2016), we also examined whether the action of fluoxetine is mediated through atropine (a muscarinic receptor antagonist) and ondansetron (a 5-HT$_3$ receptor antagonist).

Methods and Materials

Chemicals and Drugs

Fluoxetine was from Divis Pharmaceuticals Pvt. Ltd., India. The positive control serotonin hydrochloride (5-HT) and the antagonists, atropine, and ondansetron were from Sigma-Aldrich (St. Louis, MO, USA). Thiopental sodium was from Jagsonpal Pharmaceuticals Ltd. (Haryana, India). NaCl, NaHCO$_3$, glucose, KCl, CaCl$_2$, MgSO$_4$, and KH$_2$PO$_4$, the components of Krebs solution, were purchased from Merck, Co., Ltd. *(*Darmstadt, Germany*)* (K-da et al. 2020).

Tissue Preparation

Seven-week-old male ICR/Mlac mice were euthanized by intraperitoneal injection with 70 mg/kg of thiopental sodium and their hearts were removed. The abdominal cavity was opened to remove the duodenum and proximal colon. Tissues were immediately placed in an ice-cold Krebs solution gassed with 95% O$_2$ and 5% CO$_2$. After the luminal content of the intestine was cleared, the duodenum and proximal colon were cut into 1 cm segments and longitudinally suspended in a 20 mL organ bath containing oxygenated Krebs solution at 37 °C. The basal tension applied to the intestinal tissues was adjusted to 0.5 g and the tissues were equilibrated for 30 min. Smooth muscle contractions were analyzed for tone, amplitude, and frequency of contraction (% of baseline), which were recorded by the PowerLab® System (AD Instruments, Australia). The data were

analyzed by LabChart version 7. This study was guided and approved by the Animals Ethics Committee of the Prince of Songkla University, Thailand (License number MHESI 6800.11/847).

Experimental Procedure

Fluoxetine was prepared in concentrations of 0.001 0.01, 0.1, 1, 10, and 100 µM. Tissues were subjected to eight treatments of 60 µL volume. Each treatment was made up of three components of 20 µL. Distilled water (DW) was the vehicle control; 1 µM 5-HT was the positive control; the muscarinic receptor antagonist was 1 µM atropine; the 5-HT$_3$ receptor antagonist was 1 µM ondansetron; and fluoxetine was applied at each prepared concentration. Each of the three components was added to the organ bath at 5 min intervals. For simplicity and clarity, the treatments have been listed as follows, numbered one to eight: 1) DW + DW + fluoxetine, 2) DW + DW + 5-HT, 3) atropine + DW + 5-HT, 4) DW + ondansetron + 5-HT, 5) atropine + ondansetron + 5-HT, 6) atropine + DW + fluoxetine, 7) ondansetron + DW + fluoxetine, and 8) atropine + ondansetron + fluoxetine. Tissues were tested in four replicates. After tissues had been equilibrated for 30 min, the first treatment was introduced into the organ bath and the tissue was incubated for 10 min. The organ bath was then drained and refilled with Krebs solution three times to remove traces of the previous treatment. The tissue was rested for 10 min to allow the contraction to return to the baseline before testing with the next treatment. The treatments involving fluoxetine commenced at a dilution of 0.001 µM and proceeded incrementally to a final concentration of 100 µM. The procedure was repeated until the tissues had been tested in every concentration of fluoxetine.

Statistical Analysis

The results were shown as means ± standard error of the mean (SEM). Statistical comparisons between 2 groups were accomplished using the student's t-test and among multiple groups, by analysis of variance (ANOVA) and Bonferroni's test. $p < 0.05$ was the significance level for statistical tests and all data were analyzed by GraphPad Prism version 5.

Results

Effect of Fluoxetine on Small and Large Intestinal Smooth Muscle Contraction

In duodenal tissue, the amplitude of contraction was significantly suppressed when the tissue was incubated in 0.001, 10, and 100 µM fluoxetine, compared with

the control (DW; 0 µM fluoxetine) (Figure 1) ($p<0.05$, $p<0.001$ and $p<0.001$, respectively, n=6). Frequency of contraction was significantly reduced compared with the control when duodenal tissue was incubated in fluoxetine at 100 µM ($p<0.001$, n=6). In proximal colonic tissue incubated in fluoxetine at 1, 10, and 100 µM, both the amplitude ($p<0.01$, $p<0.001$, and $p<0.001$, respectively) and frequency of contractions ($p<0.05$, $p<0.001$, and $p<0.001$, respectively, n=6) were significantly lower when compared with the control (DW; 0 µM fluoxetine). In contrast, the amplitude of contraction in proximal colonic tissue was significantly increased after the application of fluoxetine at 0.001 µM compared with the control ($p<0.01$, n=6). The frequency of contraction did not change.

The results indicated that a high concentration of fluoxetine (100 µM) could reduce the amplitude and frequency of contractions in both the duodenum and proximal colon, but a low concentration of fluoxetine (0.001 µM) could increase the amplitude of contraction in the proximal colon.

MECHANISMS OF ACTION OF FLUOXETINE ON SMALL AND LARGE INTESTINAL SMOOTH MUSCLE CONTRACTION

Since Afzal et al. (2018) demonstrated that the effect of fluoxetine on intestinal contraction was related to 5-HT, we investigated the mechanism of action of fluoxetine on duodenal and proximal colonic contractions through the effect of a 5-HT and 5-HT$_3$ receptor antagonist (ondansetron).

First, we confirmed the direct effects on intestinal contraction of 5-HT at 1 µM. After the application of 5-HT at 1 µM, the tone of duodenal contraction was significantly higher compared with the control ($p<0.05$, n=6) (Figure 2(A)). In contrast, in the proximal colonic tissue, the tone of contraction did not change (Figure 2(B)). In both duodenal and proximal colonic tissue ($p<0.001$ and $p<0.05$, n=6), the amplitude of contraction was significantly reduced compared with the control (Figure 2(C) and 2(D)). The frequency of contractions when compared with the control was significantly reduced only in the duodenal tissue while the frequency of contraction in the proximal colonic tissue did not change (Figure 2(E) and 2(F)).

In addition, we investigated the effect of serotonin at 1 µM after pre-incubating tissues in atropine at 1 µM. In duodenal tissue pre-incubated in atropine and exposed to serotonin, the tone of contraction did not change. In proximal colonic tissue, the tone of contraction was significantly lower when compared with the initial baseline ($p<0.05$, n=6) (Figure 3(A) and 3(B)). The

amplitude of contraction was significantly lower than the initial baseline in both duodenal and proximal colonic tissue ($p<0.01$ and $p<0.01$, n=6) (Figure 3(C) and 3(D)), but the frequency of contraction did not change in either tissue (Figure 3(E) and 3(F)). The results indicated that pre-incubation with atropine could prevent 5-HT-induced changes in the tone and frequency of contractions in the duodenum but not in the proximal colon.

We also tested the contribution of the 5-HT$_3$ receptor in 5-HT-mediated change in intestinal contraction. We found that duodenal tissue pre-incubated with ondansetron at 1 µM before treatment with 5-HT at 1 µM showed no change in the tone of contraction when compared with the initial baseline, but proximal colonic tissue showed a significant reduction in tone ($p<0.05$, n=6) (Figure 4(A) and 4(B)). The amplitude of contraction was significantly reduced when compared with the initial baseline in both tissues ($p<0.01$ and $p<0.05$, n=6) (Figure 4(C) and 4(D)). The frequency of contraction did not change in either tissue (Figure 4(E) and 4(F)). These results suggested that ondansetron could inhibit the action of 5-HT on the tone and frequency of contraction in the duodenum but not in the proximal colon.

Duodenal tissue treated with 1 µM serotonin after pre-incubation with 1 µM atropine and 1 µM ondansetron showed no change in the tone of contraction, but proximal colonic tissue in the same condition showed a significant reduction in the tone of contraction when compared with the initial baseline ($p<0.001$, n=6) (Figure 5(A) and 5(B)). The amplitude and frequency of contractions did not change in either the duodenal or proximal colonic tissue (Figure 5C), 5(D), 5(E), and 5(F)). The results suggested that the combined effect of atropine and ondansetron could prevent 5-HT-induced reductions in the amplitude of contraction in both the duodenum and proximal colon.

Since we had established that fluoxetine at 0.001 µM could increase the amplitude of contraction in proximal colonic tissue but not in the duodenal tissue, we determined the direct effect of fluoxetine at 0.001 µM on the tone, amplitude, and frequency of contractions in the proximal colon. In the proximal colonic tissue, the tone of contraction was significantly higher when compared with the control ($p<0.05$, n=6) (Figure 6(A)) and the amplitude of contraction was slightly higher when compared with the control ($p>0.05$) (Figure 6(B)), but the frequency of contraction showed no change (Figure 6(C)). The result indicated that fluoxetine at 0.001 µM could increase the tone of contraction in the proximal colon.

To determine whether the effect of fluoxetine is mediated via muscarinic and 5-HT$_3$ receptor activation, we investigated the effect on proximal colonic tissue of

fluoxetine treatment after pre-incubation with atropine and ondansetron. After pre-incubation with atropine at 1 µM or ondansetron at 1 µM, the tone of contraction was significantly lower than it was after treatment with fluoxetine at 0.001 µM without pre-incubation ($p<0.05$, n=4-6) (Figure 6(D)). The amplitude of contraction in proximal colonic tissue was significantly lower after

incubation with ondansetron prior to treatment with fluoxetine at 0.001 µM ($p<0.05$, n=4-6) (Figure 6(E)). However, the frequency of contraction was not affected by fluoxetine at 0.001 µM and pre-incubation with the antagonist (Figure 6(F)). Our findings suggested that fluoxetine-induced change in the amplitude of proximal colonic contraction might be mediated through muscarinic and 5-HT$_3$ receptors.
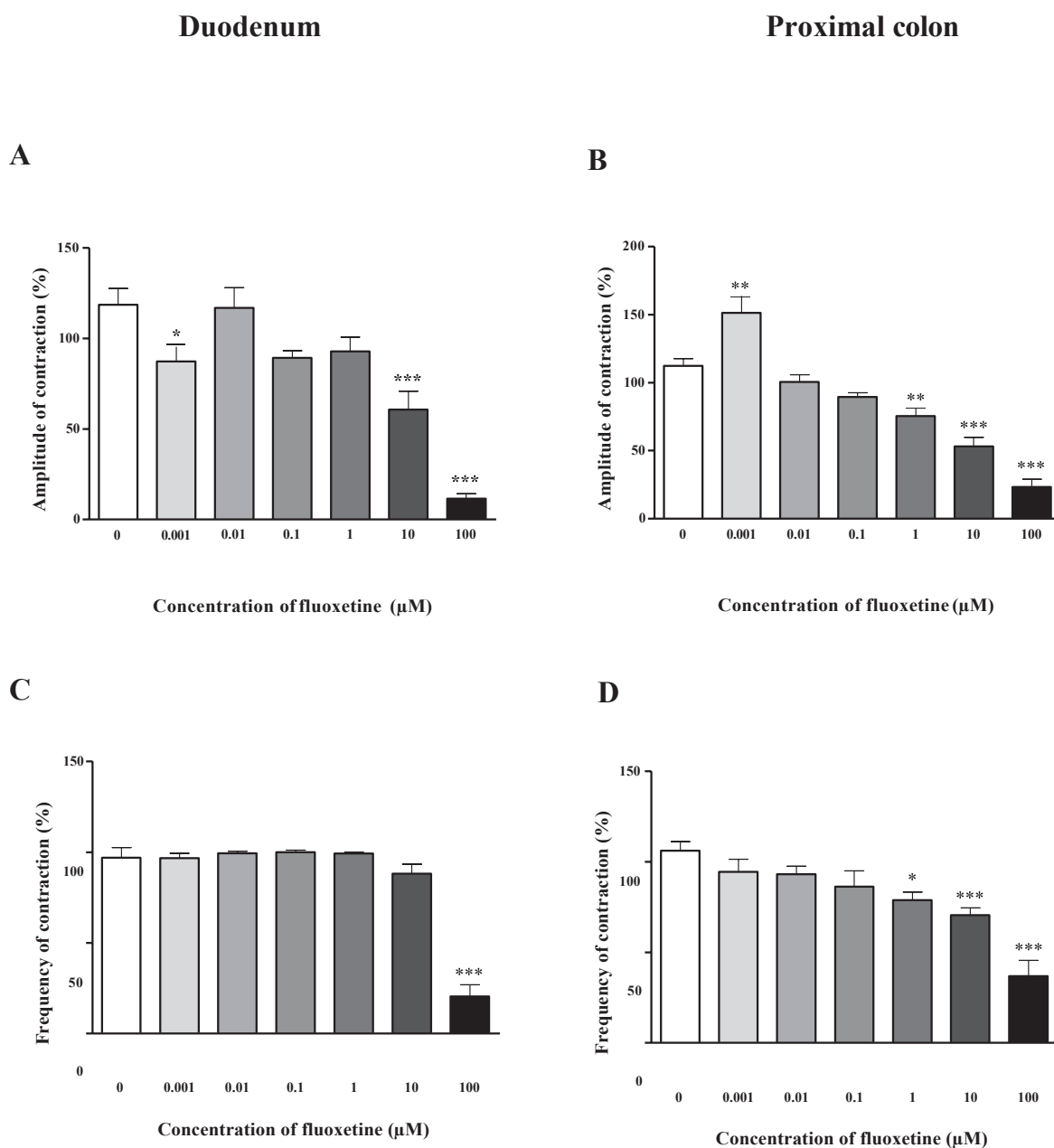
Duodenum

Proximal colon

FIGURE 1. The effects of 0.001, 0.01, 0.1, 1, 10, and 100 µM fluoxetine on longitudinal smooth muscle contractions were investigated in duodenal and proximal colonic tissue. The charts show the effects of fluoxetine on (A) the amplitude and (C) the frequency of duodenal smooth muscle contraction, and on (B) the amplitude and (D) the frequency of proximal colonic smooth muscle contraction. Data were presented as means ± SEM (n=6). Symbols show significant differences from 0 µM fluoxetine (*, **, and *** mean $p< 0.05$, 0.01, and 0.001)

Duodenum

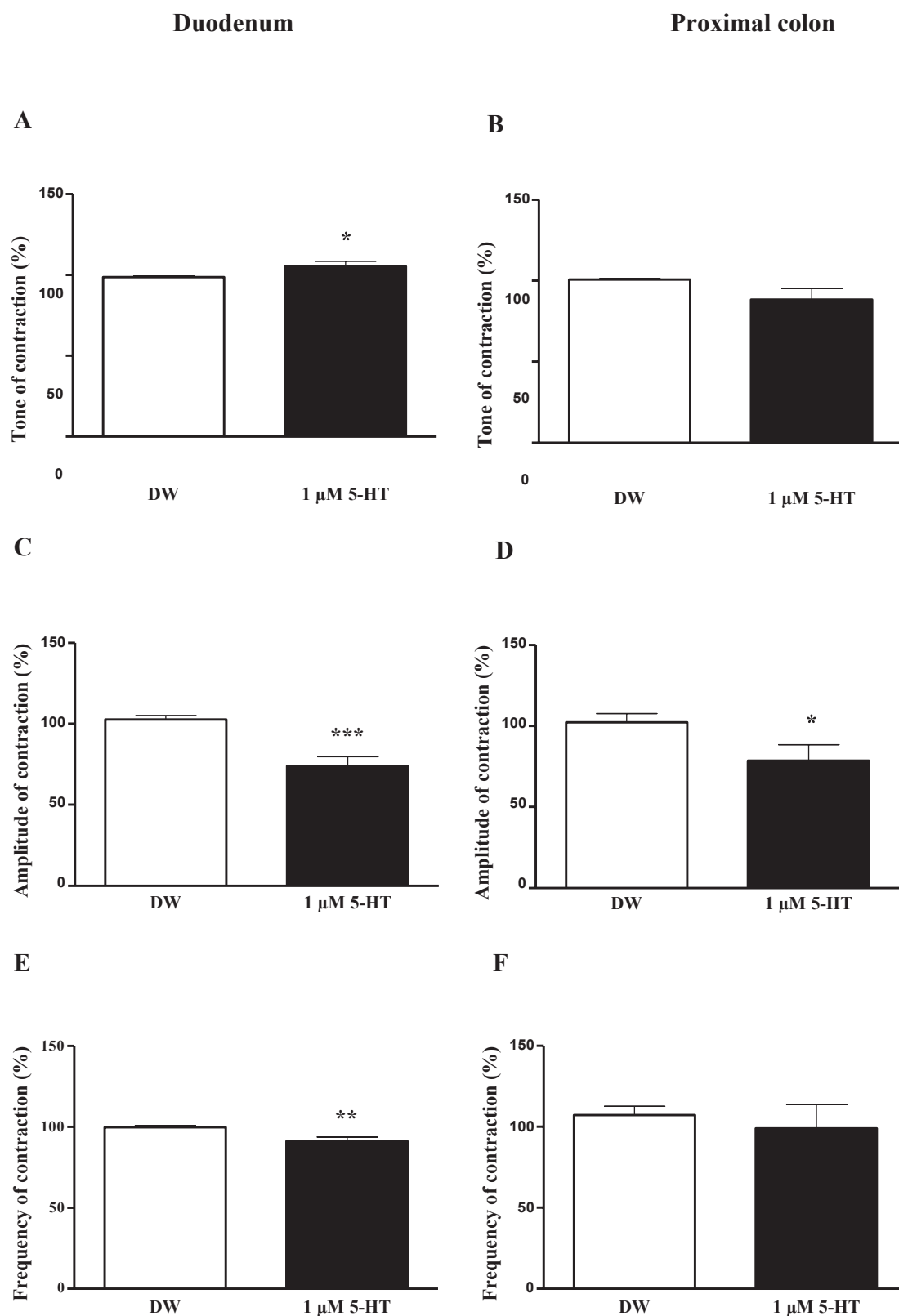**Proximal colon**

A

B

C

D

E

F

FIGURE 2. The effects of 1 μM 5-HT are shown on (A) the tone, (C) the amplitude, and (E) the frequency of duodenal smooth muscle contraction, and on (B) the tone, (D) the amplitude, and (F) the frequency of proximal colonic smooth muscle contraction. Data were presented as means ± SEM (n=6). Symbols show significant differences from DW (*, **, and *** mean $p < 0.05$, 0.01, and 0.001)

Duodenum

Proximal colon

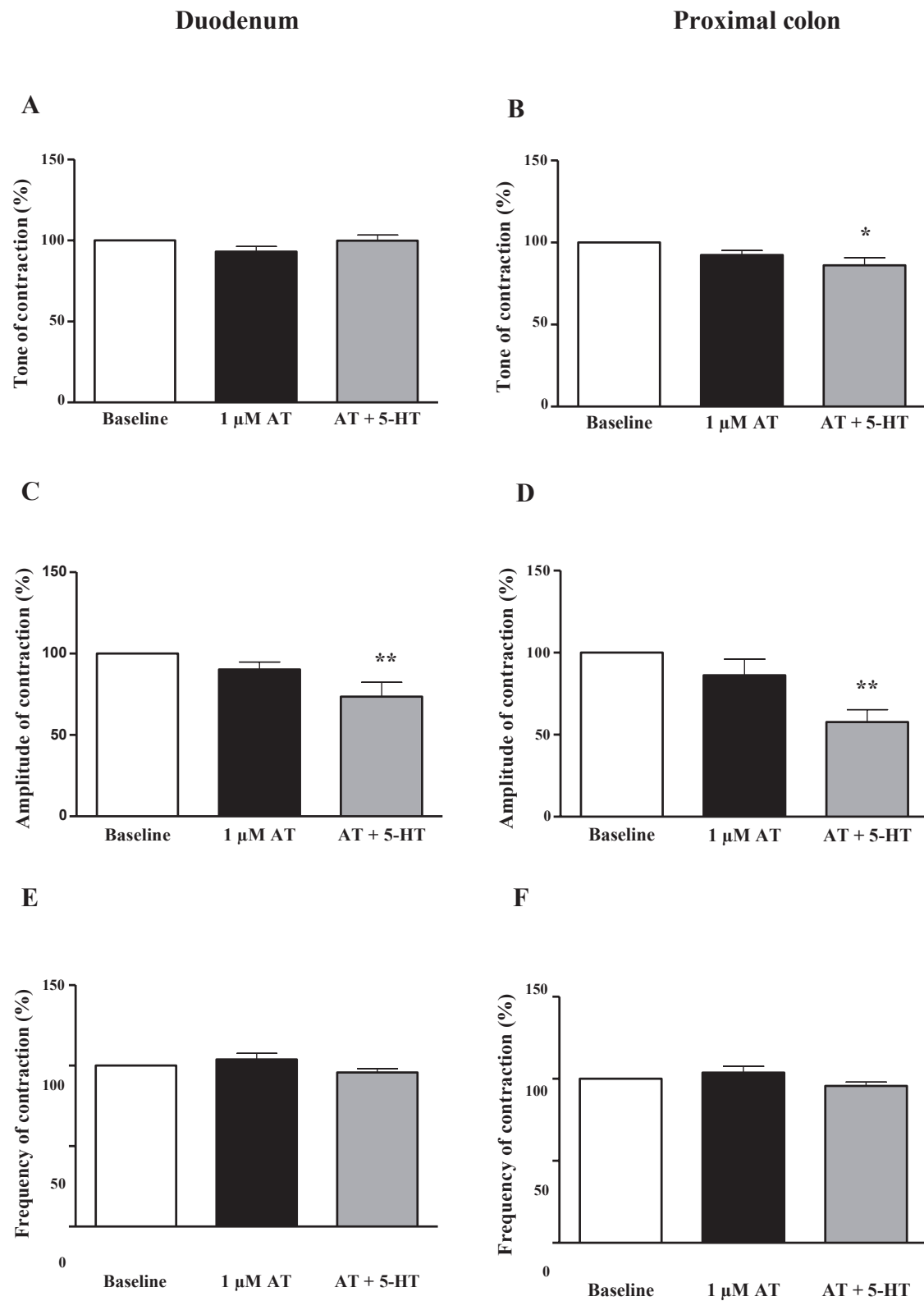FIGURE 3. The charts show the effects of 1 μM atropine and 1 μM atropine + 1 μM 5-HT on (A) the tone, (C) the amplitude, and (E) the frequency of duodenal smooth muscle contraction, and on (B) the tone, (D) the amplitude, and (F) the frequency of proximal colonic smooth muscle contraction. Data were presented as means ± SEM (n=6). Symbols show significant differences from the initial baseline (* and ** mean $p < 0.05$ and 0.01)

Duodenum

Proximal colon
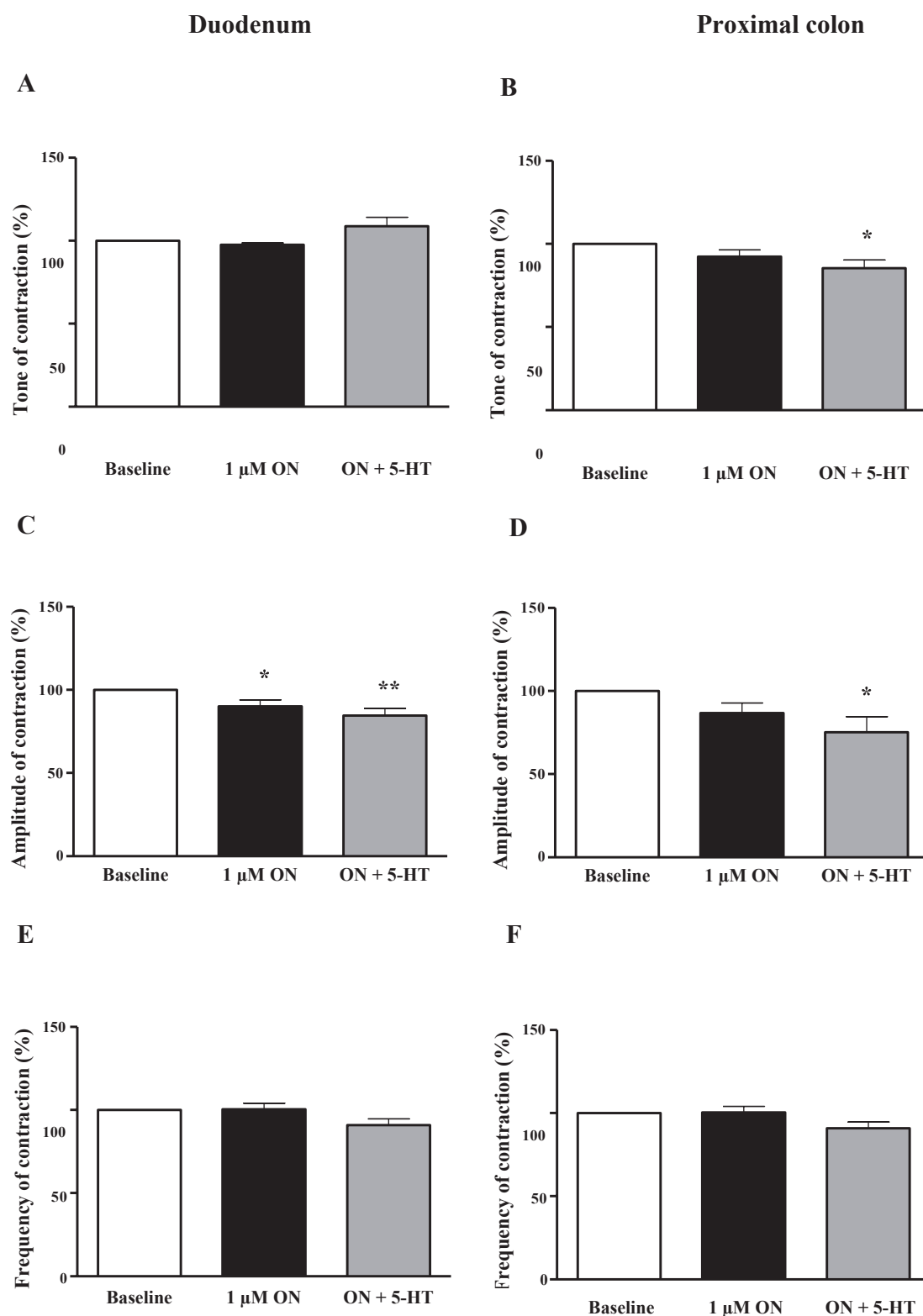
A

B

C

D

E

F

FIGURE 4. Effects are shown of 1 μM ondansetron and 1 μM ondansetron + 1 μM 5-HT on (A) the tone, (C) the amplitude, and (E) the frequency of duodenal smooth muscle contraction, and on (B) the tone, (D) the amplitude, and (F) the frequency of proximal colonic smooth muscle contraction. Data were presented as means ± SEM (n=6). Symbols show significant differences from the initial baseline (* and ** mean $p < 0.05$ and 0.01)
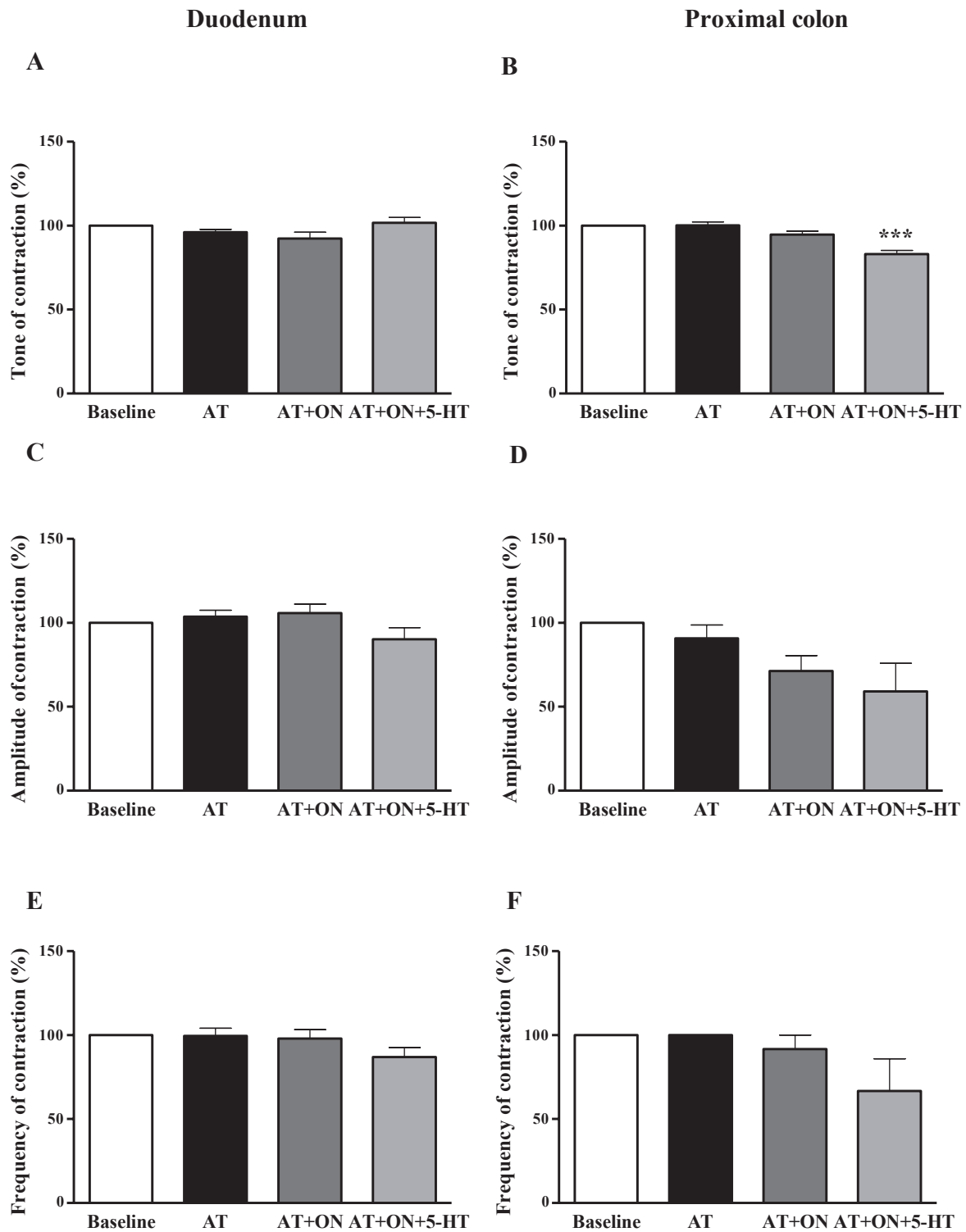
Duodenum

Proximal colon

FIGURE 5. Effects are shown of 1 μM atropine, 1 μM atropine + 1 μM ondansetron, and 1 μM atropine + 1 μM ondansetron + 1 μM 5-HT on (A) the tone, (C) the amplitude, and (E) the frequency of duodenal smooth muscle contraction, and on (B) the tone, (D) the amplitude, and (F) the frequency of proximal colonic smooth muscle contraction. Data were presented as means ± SEM (n=6). Symbols show significant differences from the initial baseline (*** means $p < 0.001$)
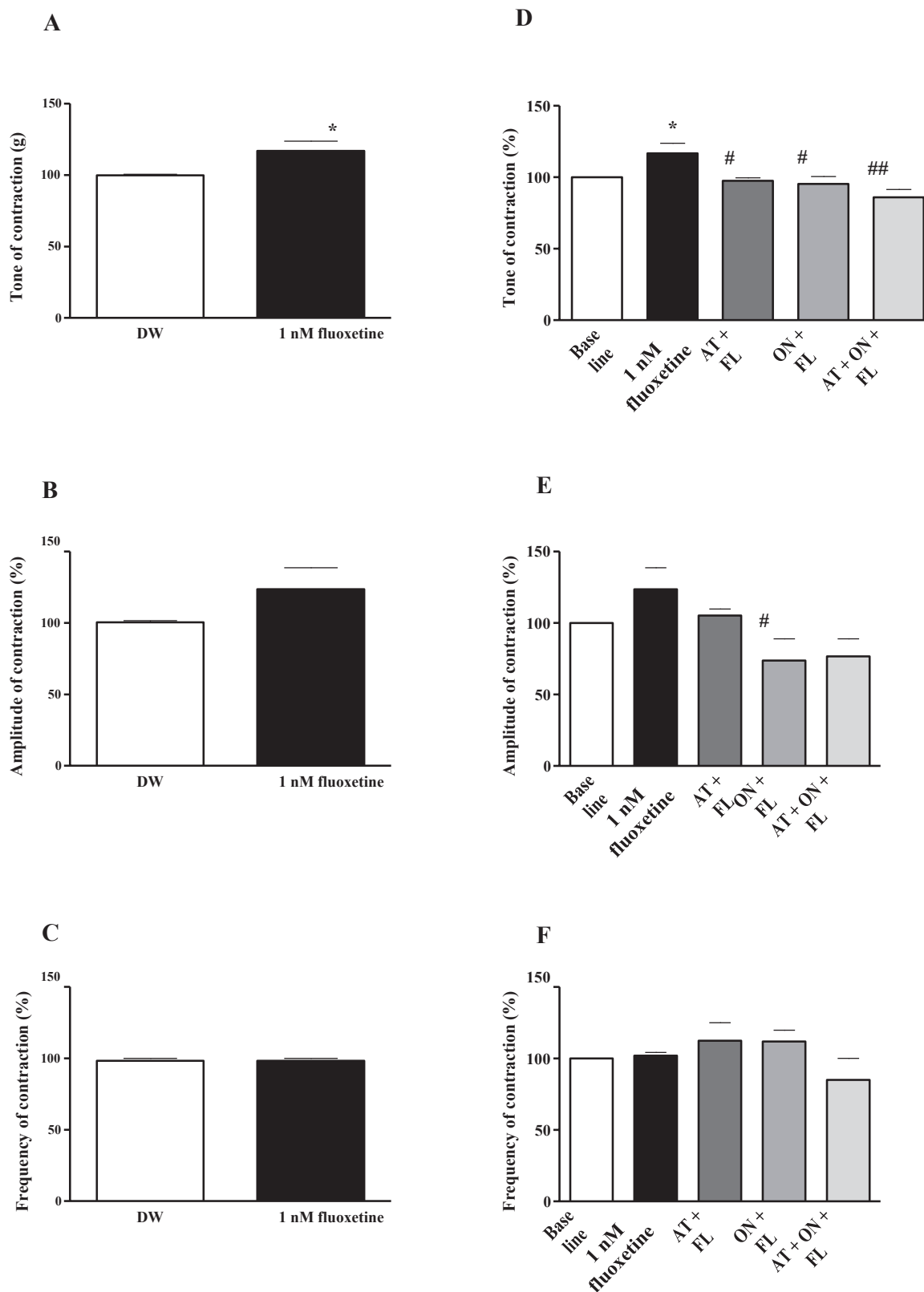
FIGURE 6. Effects of 1 nM fluoxetine on (A) the tone, (B) the amplitude, and (C) the frequency of proximal colonic smooth muscle contraction. Data were presented as means ± SEM (n=6). Symbols show significant differences from DW (* means $p< 0.05$), and effects of 1 nM fluoxetine, 1 μM atropine + 1 nM fluoxetine, 1 μM ondansetron + 1 nM fluoxetine, and 1 μM atropine + 1 μM ondansetron + 1 nM fluoxetine on (D) the tone, (E) the amplitude, and (F) the frequency of proximal colonic smooth muscle contraction. Data were presented as means ± SEM (n= 4-6). Symbols show significant differences from the initial baseline or 1 nM fluoxetine (* means $p< 0.05$ when compared with the initial baseline, and # and ## means $p< 0.05$ and 0.01 when compared with 1 nM fluoxetine)

DISCUSSION

This study showed that high concentrations of fluoxetine (1, 10, and 100 µM) reduced the amplitude and frequency of contractions in duodenal and proximal colonic tissue but a low concentration of fluoxetine (0.001 µM) increased the amplitude of contraction in the proximal colon. These results were consistent with those of an earlier report (Mawe & Hoffman 2013). Another previous study reported that fluoxetine specifically inhibited the action of SERT, leading to an increase in the extracellular concentration of 5-HT in the gastrointestinal mucosa (Kannen et al. 2011). However, Gwynne et al. (2014) demonstrated that the effects of fluoxetine may be due to a concentration-dependent effect on the properties of the 5-HT transporter itself since high concentrations of fluoxetine inhibited reuptake of 5-HT. In this condition, the diffusion of extracellular 5-HT could activate inhibitory 5-HT$_{1A}$ receptors, which suppress contractions. In contrast, low concentrations of fluoxetine might remain highly localized and less effective at inhibiting SERT. In this condition, the diffusion of extracellular 5-HT to the enteric neurons could be limited and 5-HT would then only activate 5-HT$_3$ receptors, which stimulate contractions.

This study found that 1 µM 5-HT increased the tone of contraction while decreasing the amplitude and frequency of contractions in duodenal tissue. Our findings support the proposition that 5-HT might play a primary role in the initiation of propulsive motility in the gastrointestinal system (Mawe & Hoffman 2013) and brought to mind the similar effect of acetylcholine (ACh) on gut movement, which might also be determined by a local release of ACh and norepinephrine in the gut wall (Burnstock 1958). Lochner and Thompson (2016) reported that atropine competitively antagonized the action of 5-HT$_3$ receptors and Gwynne et al. (2014) reported the effect of the 5-HT$_3$ receptor antagonist, ondansetron. Our study involving ondansetron confirmed the role of the 5-HT$_3$ receptor in modulating intestinal contraction. Some 5-HT receptor subtypes, including the 5-HT$_{1A}$ receptor, may exert inhibition on transmitter release, which possibly induced smooth muscle relaxation in the intestine (De Ponti 2004).

Sohel et al. (2020) reported that the most common gastro-intestinal side effect of fluoxetine is diarrhea. However, another study reported that some patients who had used fluoxetine also suffered from constipation (Kashani et al. 2017). According to our study, fluoxetine at 0.001 µM stimulated colonic contraction, which would lead to diarrhea in a manner consistent with the common gastro-intestinal side effects of fluoxetine (Sohel et al. 2020). On the other hand, fluoxetine at 1, 10, and 100 µM suppressed colonic contractions, which would probably lead to constipation in a manner consistent with the study of Afzal et al. (2018). Afzal et al. (2018) demonstrated that the higher the concentration of fluoxetine, the lower the contractility of the intestinal smooth muscle, which would lead to constipation. Therefore, our study indicated that a fluoxetine concentration of 0.001 µM may increase extracellular 5-HT concentrations in the intestinal mucosa enough to activate 5-HT$_3$ receptors, which were antagonized by ondansetron and the muscarinic receptor antagonist, atropine.

CONCLUSION

Our study shows that a low concentration of fluoxetine (0.001 µM) had a direct effect on intestinal contraction. This treatment increased the amplitude of contraction in proximal colonic tissue but decreased the amplitude in duodenal tissue. In contrast, high concentrations of fluoxetine (1, 10, and 100 µM) suppressed both duodenal and colonic contractions. The increase in the amplitude of contraction in the proximal colon, which would cause diarrhea, was mediated via 5-HT$_3$ and muscarinic receptors in the intestinal wall. These findings might explain the contradictory symptoms of the gastro-intestinal side effects of fluoxetine treatment.

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