The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vitro Experiments

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ITEM		
Title	1	Different distributions of ICCs and PDGFRα$^+$ cells in colonic SIP syncytium in mouse
Abstract	2	To investigate the distribution and function of the two interstitial cells that Cajal interstitial cells (ICCs) and platelet-derived growth factor receptor-α positive (PDGFRα$^+$) cells in proximal and distal colon. Colonic migrating motor complexes (CMMCs), smooth muscle spontaneous contractile experiments, intracellular recordings and western blotting analysis were used in this study. The amplitude of stimulation-induced NO/ICC-dependent slow inhibitory junctional potentials (sIJPs) by intracellular recordings from the smooth muscles in the proximal colon was larger than that in the distal colon, while the amplitude of electric field stimulation-induced purinergic/PDGFRα-dependent fast inhibitory junctional potentials (fIJPs) in the distal colon was larger than that in the proximal colon. Consistently, protein expression levels of c-Kit and Ano1 in the proximal colon were much higher, however, protein expression levels of PDGFRα and SK3 in distal colon were much higher. The ICCs mainly distribute in proximal colon and more PDGFRα$^+$cells are in distal colon, which generates a pressure gradient at both ends of colon to propel the feces to the anus.

INTRODUCTION		
Background	3	In the gastrointestinal (GI) tract, SIP syncytium consists of ICCs, PDGFRα$^+$ cells and smooth muscle cells (SMCs). ICC and PDGFRα$^+$ cell, are involved in the smooth muscle contraction. Remodeling or damaging of these cells can result in a variety of motor disorders. Cells in the SIP syncytium express many kinds of receptors and ion channels, and conductance changes in any type of cell can induce the excitability or relaxation of smooth muscle. Although there have been several studies of ICCs and PDGFRα$^+$ cells in the GI tract regarding their location, morphology, function and more, most studies have focused on the small intestine and stomach, with few concerning the colon.
Objectives	4	Adult male ICR mice were obtained from the Experimental Animal Centerof Shanghai Jiao Tong University School of Medicine. The mice, aged 35 days and weighing approximately 30 g. Based on these previous reports, we sought to characterize the mechanism of ENS and SIP in the regulation of colon motility, especially distributions of ICCs and PDGFRα$^+$ cells in proximal and distal colon.

METHODS		
Ethical statement	5	This research rigorously complied with the rules of the Guide for the Care and Use of Laboratory Animals of the Science and Technology Commission of the P.R.C. (STCC Publication No. 2, revised 1988). The protocol was approved by the Committee on the Ethics of Animal Experiments of Shanghai Jiao Tong University School of Medicine (Permit Number: Hu 686-2009). All operations were performed under anesthesia using isopentane, and every manipulation of the experimental animals was performed while simultaneously attempting to maximally relieve any suffering.
Study design	6	All animals were obtained from the Experimental Animal Center of Shanghai Jiao Tong University School of Medicine. Mice were sacrificed under general anesthesia induced by inhalant isoflurane overdose followed by cervical dislocation. a. CMMC experiments. The mechanical activity of the CMMCs was recorded the colonic contraction activity using an isometric force transducer linked to an amplifier device. b. smooth muscle tissue and isometric tension measurement. c. Western blot analysis. d. Intracellular microelectrode recording and electrical stimulation.

Experimental procedure 7 Adult male ICR mice were obtained from the Experimental Animal Centerof Shanghai Jiao Tong University School of Medicine. The mice, aged 35 days and weighing approximately 30 g, were housed at 22 ℃ under a 12 h light/dark cycle with free access to water and food. Mice were sacrificed under general anesthesia induced by inhalant isoflurane overdose followed by cervical dislocation. Then, the abdomen was opened along the ventral midline, and the colon was exposed, removed quickly and placed into Krebs solution continuously bubbling with a carbonated mixture (5% CO_2 and 95% O_2). The mesentery was carefully removed along the boundary line of the enterocoel under a dissecting microscope. All fecal pellets in the colon were artificially expelled with a 1 ml injector; this procedure was repeated to expel every pellet. This procedure must be performed with care to minimize intestinal damage. The colon was cut along the mesentery, which is on the colonic circular axis; the pellets were flushed out with Krebs; the colon was pinned to a Sylgard dish with the mucosa facing upwards; and the mucosa and submucosa were removed carefully under a dissecting microscope. Smooth muscle strips (approximately 2 mm×8 mm) were obtained by cutting along the circular axis from the fresh smooth muscle tissue.	
Experimental animals 8 All animals were obtained from the Experimental Animal Center of Shanghai Jiao Tong University School of Medicine. Adult male ICR mice were aged 35 days and weighing approximately 30 g, were housed at 22 ℃ under a 12 h light/dark cycle with free access to water and food. Mice were sacrificed under general anesthesia induced by inhalant isoflurane overdose followed by cervical dislocation. All operations were performed under anesthesia using isopentane, and every manipulation of the experimental animals was performed while simultaneously attempting to maximally relieve any suffering.	

Housing and husbandry **9** Adult male ICR mice were aged 35 days and weighing approximately 30 g, were housed at 22 ℃ under a 12 h light/dark cycle with free access to water and food.

Sample size **10** In this study, about 48 animals were used in total, and 8 to 12 animals were used in each group of the control and experimental groups to obtain statistically different data.

Animal experimental grouping **11** The animals used in the experiment were randomized into groups.

Experimental outcomes **12** our findings in the present study provide clear evidence that the ENS regulates colonic motility through cholinergic and nitrergic neurons regulating the spontaneous rhythmic pacemaker activity of ICCs and through purinergic neurons mediating inhibitory effects on smooth muscle contractions by acting on P2Y1 receptors in PDGFRα$^+$ cells to suppress colonic transit. We further found that inhibitory neuromodulation had a leading role in colonic transmission, which may be, associated with the formation of fecal pellets in the colon and full absorption of water and nutrients. Furthermore, the distribution characteristics of more ICCs in the proximal and more PDGFRα$^+$ cells in the distal colon contribute to the formation of a pressure gradient from the oral to anal ends of the colon, which is associated with the roles of ENS-NO/ICCs and purine/PDGFRα$^+$ cells in the regulation of colonic motility.

Statistical methods **13** The data are described as the means ± SE. The analysis of data differences between groups was performed with one-way ANOVA, followed by Bonferroni's post-hoc testing or using Student's unpaired *t* test when needed. P-values less than 0.05 were considered to represent significant differences between groups, and n-values correspond to the number of animals that were used in the indicated experiments.

RESULTS

Baseline data **14** Before the experiment, the animal's weight was measured, and fecal traits, hair color, mental state and other health indicators were observed.

Numbers analysed **15** In the analysis of experimental data, a very small number of data with large deviations or unqualified data of experimental animals are discarded.

Outcomes and estimation **16** P-values less than 0.05 were considered to represent significant differences between groups, and n-values correspond to the number of animals that were used in the indicated experiments.

Adverse events **17** To minimize adverse reactions strictly in accordance with the standard operation of animal experiments.

DISCUSSION

Interpretation/scientific implication **18** Cells in the SIP syncytium express many kinds of receptors and ion channels, and conductance changes in any type of cell can induce the excitability or relaxation of smooth muscle. Although there have been several studies of ICCs and PDGFRα$^+$ cells in the GI tract regarding their location, morphology, function and more, most studies have focused on the small intestine and stomach, with few concerning the colon. ANO1, a very important functional protein in ICCs, is a calcium-activated chloride channel that produces pacemaker currents. Another interstitial cell, PDGFRα$^+$ cells, referred to as "fibroblast-like" cells, express specific small conductance calcium-activated potassium channels, namely SK3.

In this study, we strictly adhere to the principles of substitution, optimization and reduction of animal use (the 3R principle) to minimize animal suffering.

Generalization/transformation **19** The distribution characteristics of more ICCs in the proximal and more PDGFRα$^+$ cells in the distal colon contribute to the formation of a pressure gradient from the oral to anal ends of the colon, which is associated with the roles of ENS-NO/ICCs and purine/PDGFRα$^+$ cells in the regulation of colonic motility. Consequently, an in-depth understanding of the mechanism of colon motility may provide a new direction and target for the study of the treatment for colon motility disorder, such as IBD and IBS.

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References:

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