

Role of intracellular calcium in contraction of internal anal sphincter

NIU Wei-Xin¹, QIN Xin-Yu¹, LU Ying-Qing², SHI Nian-Ci² and WANG Cheng-Pei¹

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INTRODUCTION

Internal anal sphincter (IAS) is a continuation of the smooth circular muscle layer thickened at the rectum, innervated by vegetative nerve. IAS is a special smooth muscle, which is different from colonic smooth muscle in physiology and pharmacology^[1]. It was found that contraction of gastric smooth muscle depends on the influx of extracellular calcium and release of intracellular calcium^[2]. In present study, we observed and compared the effects of extra and intracellular calcium on the contraction of IAS and colonic smooth muscle.

MATERIALS AND METHODS

IAS and colonic smooth muscle (circular muscle) were taken from 10 patients undergoing abdomino-perineal resection of the rectal carcinoma. The strips containing muscle were approximately 1.2 cm long and 0.2 cm wide. The samples were immediately immersed in Krebs-Henseleit solution. The muscle segments were held by the extremities in the direction of the fibres and suspended in a 35mL organ bath containing Krebs-Henseleit solution at $36.5^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ and gassed with 95% oxygen-5% carbon dioxide. Segments were attached to an isometric transducer coupled to a polygraph recorder.

Responses of the strips to acetylcholine (Ach) and noradrenaline (NA) were examined using cumulative concentration responses. After this, repeated steps were made in calcium-free and

calcium solution respectively to test effects of extracellular calcium on the contraction of the muscle. Finally, ryanodine of different doses was given to the strips in solution containing calcium to study the role of intracellular calcium in contraction of the muscle.

Anal manometry was made in 32 healthy volunteers to obtain the contracting figures of anal sphincter. The average age was 47 years (ranging from 33-61). The manometry consisted of a perfused open-ended polyethylene catheter (inside diameter 1mm) attached to an external transducer (CYS, China), and a micro computer (Laser-310).

Statistical results were compared using Student's test. $P < 0.05$ was considered to be significant.

RESULTS

Contracting frequency of colonic smooth muscle and IAS

In vitro spontaneous contraction of 7-12 and 11-17 cycles per min (cpm) could be seen in colonic smooth muscle and IAS respectively. The contracting frequency of anal sphincter from the manometric figures was 12 cpm-18 cpm under the resting condition in these volunteers.

Effects of Ach and NA on colonic smooth muscle and IAS

Ach had a dose-dependent relation with contraction of colonic smooth muscles and had no effect on internal anal sphincter. NA had a dose-dependent relation with contraction of internal anal sphincter and had no effect on colonic smooth muscles (Table 1).

Contracting effects of extracellular calcium on colonic smooth muscle and IAS

Significant difference was found in contractions of colonic smooth muscles in calcium and calcium-free solutions, but no difference was found in contraction of IAS in calcium and calcium-free solutions (Table 2).

Effects of ryanodine on contraction of colonic smooth muscle and IAS

Ryanodine had a remarkably inhibiting effect on contractions of IAS, but had no effect on contractions of colonic smooth muscles (Table 3).

¹Department of General Surgery, Zhongshan Hospital, Shanghai Medical University, Shanghai 200032, China

²Laboratory of Basic Pharmacology, Shanghai Medical University, Shanghai 200032, China

Dr. NIU Wei-Xin, male, born on 1958-01-26 in Hefei City, Anhui Province, Han nationality, graduated from Shanghai Medical University as a postgraduate in 1991, associate professor of surgical department, having 8 papers published.

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Correspondence to: Dr. NIU Wei-Xin, Surgical Department, Zhongshan Hospital, Shanghai Medical University, 136 Yi-xue-yuan road, Shanghai 200032, China.

Tel. +86 • 21 • 64041990 Ext. 2810 or 2640

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Table 1 Dose-response correlation of Ach and NA in colonic smooth muscle and IAS ($\bar{x} \pm s$, $n = 10$)

Ach (mol/L)	Colonic smooth muscle		Internal anal sphincter (IAS)	
	Contraction strength (g)		NA (mol/L)	Contraction strength (g)
10^{-6}	1.2 ± 0.39		10^{-6}	0.70 ± 0.23
10^{-5}	2.4 ± 0.41		5×10^{-6}	1.34 ± 0.41
10^{-4}	4.1 ± 1.20		10^{-5}	1.74 ± 0.41
10^{-3}	6.4 ± 1.60		5×10^{-5}	2.20 ± 0.44
10^{-2}	9.3 ± 1.75		10^{-4}	2.68 ± 0.45

Table 2 Contraction of colonic smooth muscle and IAS in different solutions ($\bar{x} \pm s$, $n = 10$)

Solution	Contraction strength (g)	
	Colonic smooth muscle	Internal anal sphincter
Ca ²⁺ solution	8.30 ± 2.01	2.44 ± 1.20
Ca ²⁺ -free solution	4.27 ± 0.91^a	1.84 ± 0.77

^a $P < 0.01$, $t = 3.72$, compared with Ca²⁺ containing solution.

Table 3 Inhibiting effect of ryandine on colonic smooth muscle and IAS ($\bar{x} \pm s$, $n = 10$)

Ryanodine ($\mu\text{mol/L}$)	Percentage of contractile strength without ryanodine (%)	
	Colonic smooth muscle	Internal anal sphincter (IAS)
0.1 ^a	85 ± 17.9	91 ± 11
1.0	87 ± 13	71 ± 24^b
10.0	85 ± 14	57 ± 20^c

^b $P < 0.05$, $t = 2.48$; ^c $P < 0.01$, $t = 3.61$; compared with a.

DISCUSSION

Smooth muscle of gut is innervated by cholinergic and adrenergic nerves. Acetylcholine is effective in contracting colonic smooth muscles, whereas epinephrine inhibits it. It is these nerves that regulate and control the movement of the smooth muscles of digestive tract.

The contracting frequency of IAS is basically in accordance with anal sphincter in the volunteers. The contracting frequency of IAS is higher than that of colonic circular muscle. It is possibly related to the maintenance of continence because anal resting pressure is mainly produced by contraction of IAS^[3].

It is well known that contraction of muscular cell is associated with Ca²⁺, and contraction of

colonic smooth muscle is chiefly produced by influx of Ca²⁺. From present study we found that contraction of colonic smooth muscle was weakened by about 50% when no extracellular calcium exists, whereas contraction of IAS was not affected. It is suggested that releases of Ca²⁺ from intracellular storage may occur at the time when IAS contracts.

Ryanodine is an alkaloid extracted from rhizome of *Ryania speciosa* Vahl. It has been found in animal experiments that when Ryanodine is combined with Ryanodin receptor of sarcoplasmic reticulum in smooth muscular cells, contraction of the muscular cells will be prevented because Ca²⁺ storage is exhausted after release of Ca²⁺ from it^[4-7]. In the present study we found that contraction of IAS induced by agonist was significantly inhibited in contrast with colonic smooth muscle when Ryanodin was given. It is indicated that release of intracellular calcium may play an important role in contraction of IAS. IAS is a special smooth muscle, being principally responsible for the generation and maintenance of resting anal canal pressure^[3]. It is still unknown whether the contracting property of IAS is associated with the release of intracellular calcium. Further studies should be made.

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