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Full paper

Characterizations of the α_1-adrenoceptor subtypes mediating contractions of the human internal anal sphincter

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ABSTRACT

Human internal anal sphincter (IAS) is contracted by α_1-adrenoceptor stimulation and thus α_1-adrenoceptor agonists may be useful in treating fecal incontinence. This study characterizes the contribution of α_1-adrenoceptor subtypes in contraction of human IAS and to investigate the age-related risk of patients with fecal incontinence. IAS and inferior mesenteric artery (IMA), as a predictor of systemic arterial pressure, were obtained from 11 patients. Both muscle strips were assessed by isometric-contraction experiments using phenylephrine, further in IAS, in the presence of various subtype selective α_1-adrenoceptor antagonists. Immunohistochemistry and gene expression studies were performed in the same samples. The mean pEC_{50} values with SEM of phenylephrine in IAS (6.30 ± 0.13) were higher than those of IMA (5.60 ± 0.10). Furthermore, the age-related pEC_{50} change of IAS was observed between age <70 and ≥70 (6.58 ± 0.13 and 6.07 ± 0.16, respectively ($P < 0.05$)). In IAS, rightward shift of the concentration −response curves of phenylephrine was observed with three α_1-adrenoceptor antagonists. Each pK_B value of silodosin, BMY-7378 and prazosin was 9.36 ± 0.53, 7.28 ± 0.20 and 8.89 ± 0.12, respectively. These pK_B values and gene expression studies indicated that α_{1A}-adrenoceptor subtypes predominantly contributed to human IAS contraction.

1. Introduction

Passive fecal incontinence is defined as the involuntary loss of solid or liquid feces. It is a common symptom that causes significant distress and reduces quality of life, with a prevalence of 1.6%–15.3% (1). Passive fecal incontinence is caused by contractile dysfunction of the internal anal sphincter (IAS), which is located in the distal end of the rectum and composed of concentric layers of circular muscle tissue under involuntary control (2–4). This circular smooth muscle has an important role in maintaining maximum anal resting pressure (MRP) (5). Its contraction occurs through sympathetic nerve stimulation (6–9).

Since the 1990s, several clinical trials for pharmaceutical treatment of passive fecal incontinence have been conducted (10–14). Alpha$_1$-adrenoceptor agonists, phenylephrine and noradrenaline were tested, because it is well known to produce contractile response in human IAS *in vitro* (15, 16). In addition, topical drug administration around the anus were performed to avoid the common adverse effects; elevation of systemic blood pressure. In these studies, the administration of 30% (w/w) phenylephrine gel to the distal anal canal markedly increased MRP in patients with passive fecal incontinence but also elevated the systemic blood pressure (12). Further, topical administration of an α_{1A}-adrenoceptor agonist, L-erythro-methoxamine was characterized with higher α_{1A}-adrenoceptor activity than phenylephrine. L-erythro-methoxamine 3% (w/w) gel applied to the anal canal or rectum of healthy volunteers not only increased MRP for 5 h but also elevated systemic blood pressure in some healthy volunteers (17). Therefore, they adopted the lower concentrations, 0.3% or 1% (w/w) of L-erythro-methoxamine gel in patients with passive fecal incontinence. However, not only did the dose increase MRP for 2 h but also elevate systemic blood pressure (18). To date, there are no successful clinical reports using a topical α_1-adrenoceptor agonist without elevating blood pressure.

Abbreviations: IAS, internal anal sphincter; IMA, inferior mesenteric artery.

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The contribution of α_1-adrenoceptor subtypes have been studied in IAS smooth muscle of pig (19, 20) and sheep (21); $\alpha_{1A/L}$-adrenoceptor contracted it in the pig (20), whereas the α_{1A}- and α_{2D}-adrenoceptors contracted it in the sheep (21). However, there is no reliable information concerning α_1-adrenoceptor subtypes in human IAS. The first aim of the current study was to characterize the contribution of α_1-adrenoceptor subtypes in contraction of the human IAS; and the second aim was to investigate the epidemiological risk factors of patients with fecal incontinence.

2. Materials and methods

2.1. Ethical approval

Approval for the study was obtained from the Institutional Review Board for Clinical Research of Tokai University Hospital (No.09R153 and 08R033) and conducted in compliance with the ethical guidelines of clinical research in the implementation plan.

2.2. Patients

All patients provided written informed consent before participation. Specimens were obtained from 11 patients [8 males, 3 females; median age 70 (range 36–86) years old] that underwent abdominoperineal resection during the period from May to December in 2010 in Tokai University Hospital. The following anonymized patient information was obtained: race, age, gender, number of vaginal deliveries (if female), and the presence or absence of fecal incontinence symptoms. All patients had locally advanced rectal carcinoma, were Japanese, and presented with no symptoms of fecal incontinence at surgery. The vaginal deliveries frequency of patient case No.5, 6 and 8 were 1, 2 and 3 experiences, respectively. The Case No.4–11 patients received 40–45 Gy radiation.

2.3. Isometric contraction experiments

The fresh tissues including part of the IAS and inferior mesenteric artery (IMA) were obtained from the sites those were not affected by prior radiological treatment, which was confirmed by HE staining. The tissue was placed in ice-cold modified Krebs buffer gassed with 5% CO_2 in oxygen within 15 min of resection in operating room. The following procedure was started within an hour of the resection. Surrounding mucosa, submucosa, connective tissue and external-anal sphincter were removed from the tissues, and IAS and IMA were isolated. The IAS was dissected in the direction of the smooth muscle bundles. The IMA was dissected in the helical direction and its endothelium was removed. The IAS and IMA were cut into 12 mm × 3 mm strips. Three or more strips were obtained from individual tissues.

Strips were vertically placed in 10-mL organ baths containing a modified Krebs buffer at 37 °C and gassed with 5% CO_2 in oxygen. One end of each strip was connected to a force-displacement transducer (SB-1T, NIHON KOHDEN, Tokyo, Japan), and changes in muscle tension were measured and recorded on a pen-writing oscillograph (Rectigraph 8S, NEC-Sanei, Tokyo, Japan). The preparations were allowed to equilibrate with 10 mN of resting tension for at least 30 min at each 10 min interval wash out before starting the experiments. Strips were then stimulated with 0.1 mM phenylephrine and their contractile response was confirmed. After the confirmation, strips were immediately washed and allowed to equilibrate for at least 30 min until the tension returned to its base value.

The concentration–response curves were obtained by the cumulative addition of 1 nM to 1 mM of phenylephrine to the bathing solution. After attaining the successive concentration–response curves for phenylephrine, IAS strips were immediately washed and allowed to equilibrate both with and without α_1-adrenoceptor antagonists. The following antagonists were used: the selective α_{1A}-adrenoceptor antagonist, silodosin (3 nM); the selective α_{1D}-adrenoceptor antagonist, BMY-7378 (3 μM) and the non-subtype-selective α_1-adrenoceptor antagonist, prazosin (25 nM). Each preparation was equilibrated with the antagonist for 30 min before the next addition of phenylephrine in the manner described above. Only one concentration–response curve was obtained from each strip. Maximum contraction of strip was confirmed by application to 40 mM KCl at the end of experiment. The contractile responses were expressed in mN, and as a percentage of the maximum response for each concentration–response curve [expressed as mean ± standard error of the mean (SEM)].

Individual concentrations producing 50% maximal response (pEC_{50} values) were calculated by non-linear regression fitting to sigmoidal concentration–response curves (variable slope) by GraphPad Prism® software version 4 (GraphPad Software Inc, San Diego, USA). Individual affinity estimates (apparent pK_B values) were determined from the equation: $\{pK_B = [\log (EC_{50}$ without antagonist/EC_{50} with antagonist) − 1] − log (antagonist concentration)$\}$. The data are reported as mean ± SEM. Statistical significance of experimental observations is determined by the Student's *t*-test with the level of significance set at $P < 0.05$.

This study was limited by the relatively small number of patients, because it was difficult to obtain human tissues for laboratory studies, and could not use more concentrations of antagonists for calculate to pA_2 value.

2.4. Quantitative polymerase chain reaction

The IAS tissues were obtained before organ bath technique and stored in All-prep reagent® (Qiagen, USA) at − 20 °C. Total RNA samples were extracted using the RNeasy® kit (Qiagen) according to manufacturer instructions. These total RNA samples used for following both of quantitative polymerase chain reactions (PCR). Pre-designed primer sets, Perfect Real-Time™ primer (Takara Bio Inc, Otsu, Japan), were used for three genes, including α_{1A}-adrenoceptor (ID: HA073254), α_{1B}-adrenoceptor (ID: HA032618), α_{1D}-adrenoceptor (ID: HA093200). Quantitative PCR was performed in a 25-μL reaction volume using the SYBR® Premix Ex Taq™ kit (Takara Bio Inc). Complementary DNA (equivalent to 10 ng of total RNA) was synthesized using Super-Script II reverse transcriptase (Life Technologies, Tokyo, Japan) and mixed with Taq Universal Mix (Applied Biosystems, Tokyo, Japan), 0.3-μM primer sets and 0.3-μM TaqMan probe. Quantitative PCR was performed using DNA Engine Opticon (MJ Research, Cambridge, USA) as follows: 1 × 10 min at 95 °C, 40 cycles of denaturation (15 s at 95 °C) and annealing/extension (1 min at 60 °C). The exact copy number was determined by a standard curve of complementary DNA for each gene of plasmid DNA templates and shown as the number of transcripts per 1 ng total RNA equivalent. The data are reported as mean ± SEM. Statistical significance of the experimental observations was determined by one-way analysis of variance (ANOVA) and Dunnett's post-hoc multiple comparison test, with the level of significance set at $P < 0.05$.

2.5. Immunohistochemical observations

Tissues were fixed in Bouin's solution and were embedded in paraffin wax. After removing paraffin wax, sections (5 μm) were incubated in phosphate–buffered saline (PBS) containing 2% bovine serum albumin (BSA) with gentle agitation overnight at 4 °C. Sections were washed five times for 5 min each with PBS,

washed once with 2% BSA–PBS and then incubated with the primary polyclonal antibodies, including the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (diluted 1:500 in PBS) for 60 min at room temperature. The sections were washed five times (5 min each) with PBS and then washed once with 2% BSA–PBS, and incubated with Biotin-labelled goat anti-rabbit IgG secondary antibody (diluted 1:200 in PBS) for 30 min at room temperature. The sections were washed five times with PBS and subjected to immunohistochemical analysis. Polyclonal antibodies to human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors and secondary antibody (Acris Antibodies, Hiddenhausen, Germany) were obtained from Funakoshi Inc (Tokyo, Japan).

2.6. Drugs and solutions

Silodosin was obtained from Funakoshi Inc and phenylephrine, BMY-7378 and prazosin were obtained from Sigma–Aldrich Co LLC (Tokyo, Japan). All drugs were dissolved and serially diluted in saline. The composition of the modified Krebs buffer was (in mM): NaCl 119.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.1. Reagents were obtained from Nacalai tesque (Tokyo, Japan).

3. Results

3.1. Phenylephrine-induced contraction in human IAS and IMA smooth muscle

As expected, phenylephrine produced concentration–dependent contractile responses in both IAS and IMA (Fig. 1A and B). The mean pEC_{50} values with SEM for IAS and IMA were 6.30 ± 0.13 and 5.60 ± 0.10, respectively (Table 1), indicating that the effect of phenylephrine on the IAS contraction was more potent than that of the IMA ($P < 0.05$). It was also shown in the younger group (6.58 ± 0.13 vs 5.60 ± 0.13, $P < 0.05$). In addition, pEC_{50} values for 23 IAS-strips in the younger group (age < 70; n = 5 patients; 6.58 ± 0.13) were significantly higher than those for 26 IAS-strips in the elderly group (age ≥ 70; n = 6 patients; 6.07 ± 0.16) ($P < 0.05$) (Table 1, Fig. 1A). On the other hand, there was no significant difference in pEC_{50} values between the two groups in IMA (Table 1, Fig. 1B).

3.2. Effects of antagonists on phenylephrine-induced contraction in human IAS smooth muscle

All of the α_1-adrenoceptor antagonists shifted the phenylephrine-induced contraction curve to the right in human IAS. But the maximum responses were altered by neither of all antagonists. These pEC_{50} values of the shifted curve with antagonists were significantly changed from pEC_{50} value without antagonists ($P < 0.05$). Each mean pK_B value was calculated and listed with the published pK_B (pA_2) values in Table 2.

3.3. Immunohistochemical observations

The IAS smooth muscle was positively and strongly stained by the α_{1A}- and α_{1D}-adrenoceptors, but was weakly stained by α_{1B}-adrenoceptor (Fig. 2). Moreover, peripheral nerve bundles were stained by the α_{1A}-adrenoceptor and blood vessel intimal smooth muscle was stained by the α_{1D}-adrenoceptor.

3.4. Gene expression levels of α_1-adrenoceptor subtypes in IAS

The mRNA expressions of α_1-adrenoceptor subtypes were confirmed by quantitative PCR (Fig. 3A). The gene expression of α_{1A}- and α_{1D}-adrenoceptors in IAS was not different but that of α_{1B}-adrenoceptor was negligible ($P < 0.05$). In addition, each gene expression of the α_{1A}-adrenoceptor and α_{1D}-adrenoceptor was approximately 1.9-fold and 1.4-fold higher in the elderly group compared with that in the younger group, although there were no significant differences between them (Fig. 3B, C).

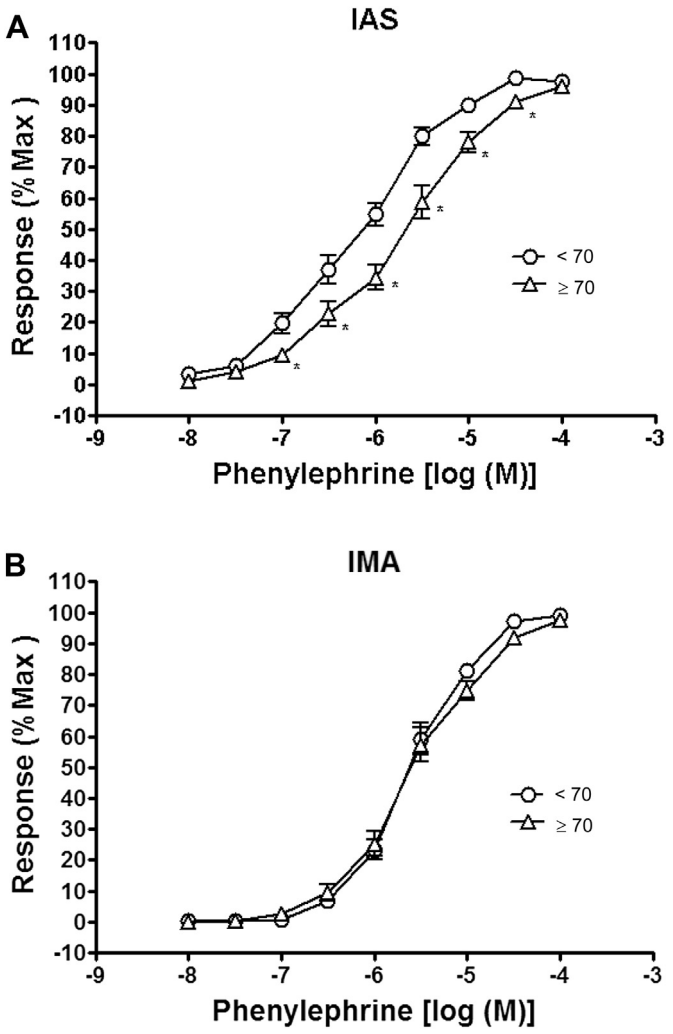

Fig. 1. Concentration–response curves for phenylephrine (1 nM–1 mM) in the smooth muscle strips from elderly and younger patients. A, the elderly subgroup (age ≥ 70; n = 26 strips from 6 patients; triangle) and the younger subgroup (age < 70; n = 23 strips from 6 patients; circle) in internal anal sphincter (IAS). B, the elderly subgroup (age ≥ 70; n = 20 strips from 5 patients; triangle) and the younger subgroup (age < 70; n = 19 strips from 5 patients; circle) in inferior mesenteric artery (IMA). Data show the mean ± SEM. Statistical significance between two concentration-response curves was determined by the Student's paired t-test after repeated measured ANOVA. Some points in concentration–response curves of the elderly are significantly higher compared with that of the younger in IAS (*$P < 0.05$).

4. Discussion

General risk factors for passive fecal incontinence include old age, female gender, high frequency vaginal delivery (1) and long term radiation therapy (13, 22). In particular, the prevalence rate of fecal incontinence increases with age in both males and females; the prevalence in those aged >60 years (23, 24) or >70 years (1) has been shown to be significantly higher compared with that in younger persons. Passive fecal incontinence is frequently caused by contractile dysfunction of IAS, which is contracted by α_1-adrenoceptor stimulation. To the best of our knowledge, no reliable

Table 1
Patient information and each of EC_{50} value for phenylephrine in human internal anal sphincter (IAS) and inferior mesenteric artery (IMA) smooth muscles.

Case no.	Gender	Age (y)	IAS pEC_{50}	IAS maximum response (mN)	IMA pEC_{50}	IMA maximum response (mN)
Elderly group (≥70)						
1	M	70	5.85	16.64	–	–
3	M	73	5.55	8.65	5.03	20.99
5	F	70	6.12	3.88	5.52	10.81
6	F	80	5.86	7.11	6.05	12.01
7	M	72	6.66	16.27	5.79	30.79
8	F	87	6.37	33.08	5.56	20.45
Younger group (<70)						
2	M	60	6.23	3.55	5.88	5.63
4	M	57	6.38	10.64	5.23	25.06
9	M	69	6.77	8.87	5.56	13.12
10	M	36	6.58	5.37	5.91	15.03
11	M	60	6.96	27.44	5.44	24.97
		Mean			Mean with SEM	
Total case		66.7	6.30 ± 0.13*	12.86 ± 2.93	5.60 ± 0.10	17.89 ± 2.47
Elderly group (≥70)		75.3	6.07 ± 0.16†	14.27 ± 4.30	5.59 ± 0.17	19.01 ± 3.61
Younger group (<70)		56.4	6.58 ± 0.13‡	11.17 ± 4.25	5.60 ± 0.13	16.76 ± 3.72

The upper part; All patients were divided into two groups by age. Data show the mean pEC_{50} of 3–6 smooth muscle strips. The lower part; Data show the mean of age and mean ± SEM of pEC_{50}. Student's paired *t*-test. *$P < 0.05$ vs IMA pEC_{50} of total case. †$P < 0.05$ vs IAS pEC_{50} of the Elderly group. ‡$P < 0.05$ vs IMA pEC_{50} of the younger group.

information about α_1-adrenoceptor subtypes in human IAS has been reported; however, topical anal treatment with α_1-adrenoceptor agonists has been used to locally contract IAS, while aiming not to elevate systemic blood pressure (12, 17, 18).

4.1. Individual difference and age-related responsiveness change in human IAS and IMA

In the present study, IAS from 11 patients, with no symptoms of fecal incontinence, contracted in a concentration–dependent manner when exposed to an α_{1A}-adrenoceptor agonist. Previous reports using noradrenaline and phenylephrine in IAS of healthy volunteers showed similar contractions, although there was no information about α_1-adrenoceptor subtypes (15, 16, 25).

Marked individual differences in pEC_{50} values were observed between IAS and IMA because patient conditions were different among individuals; for example, age [6 patients aged ≥70 years, 5 patients aged <70 years (median age 70 years)], gender, number of vaginal deliveries, and radiation therapy. Nevertheless, a significantly higher pEC_{50} value of IAS for phenylephrine than that of IMA was observed. This result suggested the possibility of differentiating effects of α_1-adrenoceptor agonist, phenylephrine on IAS compared with that on IMA according to patient conditions.

The pEC_{50} values of phenylephrine for IAS in the younger group were significantly higher than those in the elderly group (see Table 1). Furthermore, IAS sensitivity to phenylephrine was more potent than IMA in the younger group. Accordingly, phenylephrine could be a more effective therapy for fecal incontinence in the younger group. Although most patients with fecal incontinence are

Table 2
Affinity values of various antagonists of α_1-adrenoceptor in human internal anal sphincter (IAS) smooth muscles.

Antagonist	Published pK_B (pA_2) values and SEM of α_1-adorenoceptor subtypes			Mean pK_B values and SEM
	α_{1A}	α_{1B}	α_{1D}	
Silodosin	9.86 ± 0.32	7.52 ± 0.30	8.56 ± 0.45	9.36 ± 0.53
BMY-7378	6.30 ± 0.15	6.93 ± 0.25	8.80 ± 0.60	7.28 ± 0.20
Prazosin	9.20 ± 0.29	9.60 ± 0.14	9.50 ± 0.06	8.89 ± 0.12

Published pK_B (pA_2) values obtained from functional and binding studies (27–33). Mean pK_B values with SEM for antagonists obtained using phenylephrine as the agonist in human IAS (n = 8–9).

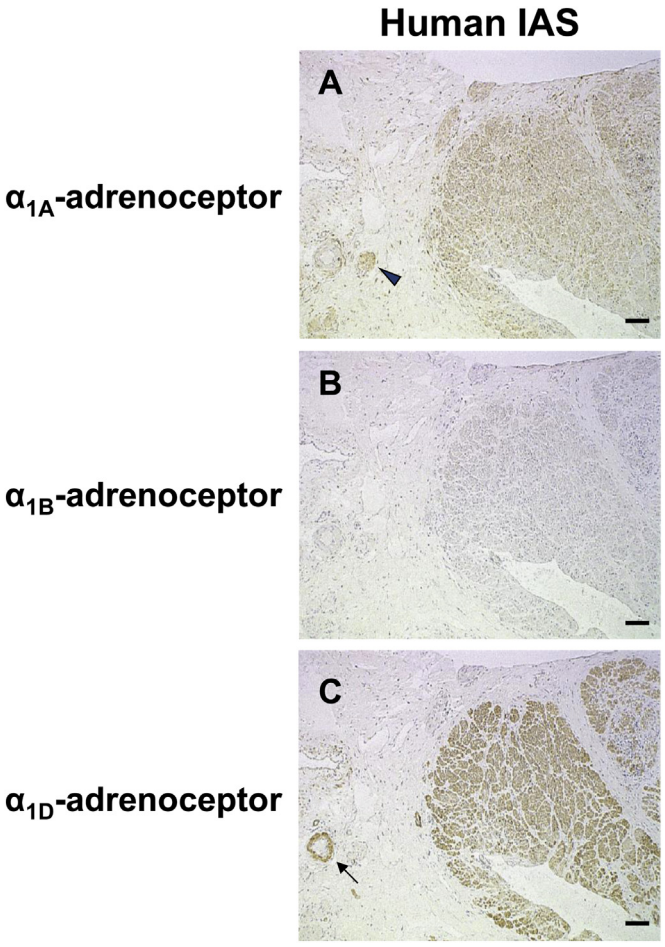

Fig. 2. Immunohistochemical analysis by staining with anti-α_{1A}-, anti-α_{1B}- and anti-α_{1D}- adrenoceptor antibodies in the human internal anal sphincter (IAS) at high magnification. Positive stain is brown. The IAS smooth muscle was positively and strongly stained by the α_{1A}- and α_{1D}-adrenoceptors, but was weakly stained by α_{1B}-adrenoceptor. Peripheral nerve bundles were stained by the α_{1A}-adrenoceptor (an arrow head in A) and blood vessel intimal smooth muscle was stained by the α_{1D}-adrenoceptor (an arrow in C). Scale bar = 100 μm.

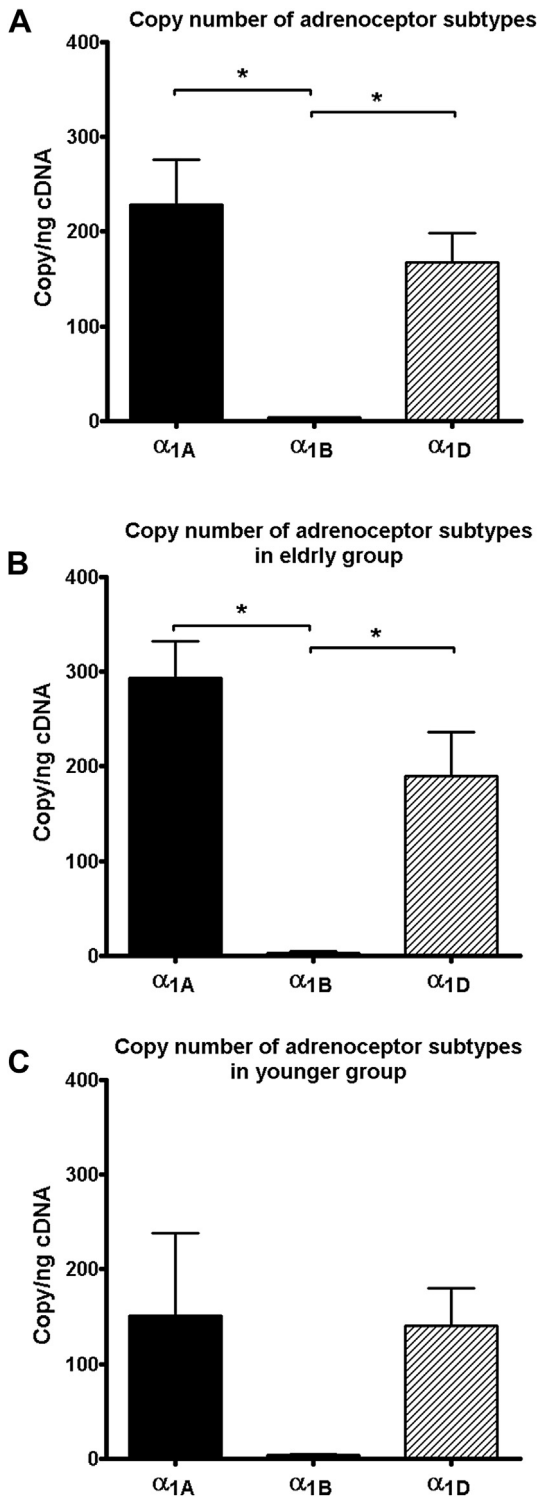

Fig. 3. Expression of α_1-adrenoceptor subtypes mRNA in the human internal anal sphincter (IAS). The data is shown in copy numbers per 1 ng complementary DNA (cDNA). A; The data show the mean ± SEM of 11 observations. B; The data show the mean ± SEM of elderly group (n = 6). C; The data show the mean ± SEM of younger group (n = 5). Statistical significance between each subtype was determined by one-way analysis of variance (ANOVA) and Dunnett's post-hoc multiple comparison test. The expression of the α_{1B}-adrenoceptor is significantly lower compared with that of the α_{1A}- and α_{1D}-adrenoceptors (*$P < 0.05$).

elderly, this information will be relevant to a specific subgroup of patients.

Each gene expression for the α_{1A}-adrenoceptor and α_{1D}-adrenoceptor in IAS of the elderly group was relatively high compared to that of the younger group (Fig. 3). However, the contraction of IAS in the elderly was weaker compared with that in the younger group (Table 1). These results suggest the possibility that reduced muscle reactivity to endogenous agonists in the elderly may result in fecal incontinence. In addition, it seems likely that the up-regulation of α_{1A}-adrenoceptor and α_{1D}-adrenoceptor gene expressions with age is not linked to the contractile function of the IAS. However, this up-regulation may be a compensatory increase in receptor gene expression caused by the decrease in reactivity.

4.2. Distribution of α_1-adrenoceptor subtypes in human IAS

The immunoreactivities for α_{1A}-adrenoceptor and α_{1D}-adrenoceptor were potent and that for α_{1B}-adrenoceptor was weak (Fig. 2). The gene expressions of α_{1A}-adrenoceptor and α_{1D}-adrenoceptor subtypes were higher than that of α_{1B}-adrenoceptor subtype, suggesting the absence of α_{1B}-adrenoceptor in human IAS (Fig. 3A). However, in the expression of each α_1-adrenoceptors may change age-dependently due to different gene expression ratios between both receptors (see Fig. 3B, C).

The pK_B value of silodosin was ranked between each published pK_B value of α_{1A}-adrenoceptor and α_{1D}-adrenoceptor. In the pK_B value of BMY-7378 is 30-fold (1.5 log units) below its affinity at α_{1D}-adrenoceptors in IAS (Table 2). On the other hand, the intermediate affinity obtained for prazosin are mediated via the α_{1A} -adrenoceptor but it is not possible to determine whether it is the high or low affinity state of this receptor that exists in the human IAS. These results suggest the possibility that α_{1A}-adrenoceptor contribute to contraction of human IAS. This possibility is supported by previous study showing that in the human urethra, the α_1-adrenoceptor subtype α_{1A} have been reported to contribute to smooth muscle contraction (34).

The IAS contraction is reported to be mediated by α_{1A}- and α_{1D}-adrenoceptor in the sheep (21) and by $\alpha_{1A/L}$-adrenoceptor in the pig (20). Thus, inter-species differences may exist in the receptor subtypes contribute to the contraction of the IAS between human and other larger mammals.

A previous study using the same approach as the present study suggested the possibility that the contraction of most arteries, including the carotid, femoral and mesenteric arteries is mediated by the α_{1A}-adrenoceptor (26). The results presented here indicate that increased systemic blood pressure may not be avoided by increasing the receptor selectivity. At present, some topical drugs those applied to anal area are developed to improve fecal incontinence. Although these drugs are applied directly to IAS and increase anal pressure, they are ultimately absorbed into the systemic circulation, resulting in detrimental elevation of systemic arterial blood pressure.

In clinical settings, any topically applied drugs with higher α_{1A}-adrenoceptor selectivity and higher activity than phenylephrine (19, 20) were also absorbed into the systemic circulation, resulting in elevated blood pressure (17, 18). These results suggest that topically administered drugs of α_{1A}-adrenoceptor agonists require some strategies preventing from the absorbance into the systemic circulation.

5. Conclusion

To the best of our knowledge this study is the first report on the differential effects of α_1-adrenoceptor subtypes in contracting the human IAS, and to reveal the corresponding gene expression levels

in detail. In addition, we examined the epidemiological risk factors of patients with fecal incontinence. This is an unpleasant disease that reduces the quality of life and dignity of sufferers in spite of ageing-related common symptoms. However, there remains the risk of systemic side effects such as elevated blood pressure, which seems particularly likely in the elderly. Treatment of fecal incontinence with α_1-adrenoceptor agonists requires a strategy to minimize the risk of elevated blood pressure among elder patients.

Conflicts of interest

There is no conflict of interest in this study.

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References

(1) Omar MI, Alexander CE. Drug treatment for faecal incontinence in adults. Cochrane Database Syst Rev. 2013;6.
(2) Bhardwaj R, Vaizey CJ, Boulos PB, Hoyle CH. Neuromyogenic properties of the internal anal sphincter: therapeutic rationale for anal fissures. Gut.. 2000;46:861–868.
(3) Bharucha AE. Outcome measures for faecal incontinence: anorectal structure and function. Gastroenterology.. 2004;126(Suppl. 1):S90–S98.
(4) Mills K, Chess-Williams R. Pharmacology of the internal anal sphincter and its relevance to faecal incontinence. Auton Autacoid Pharmacol. 2009;29:85–95.
(5) Lestar B, Pennickx F, Kerremans R. The composition of anal basal pressure. An in vivo and in vitro study in man. Int J Colorectal Dis. 1989;4:118–122.
(6) O'Kelly T, Brading A, Mortensen N. Nerve mediated relaxation of the human internal anal sphincter: the role of nitric oxide. Gut.. 1993;34:689–693.
(7) Moszkowicz D, Peschaud F, Bessede T, Benoit G, Alsaid B. Internal anal sphincter parasympathetic-nitrergic and sympathetic-adrenergic innervation: a 3-dimensional morphological and functional analysis. Dis Colon Rectum.. 2012;55:473–481.
(8) Singh J, Rattan S. Bioengineered human IAS reconstructs with functional and molecular properties similar to intact IAS. Am J Physiol Gastrointest Liver Physiol. 2012;303:G713–G722.
(9) Opazo A, Aguirre E, Saldaña E, Fantova MJ, Clavé P. Patterns of impaired internal anal sphincter activity in patients with anal fissure. Colorectal Dis. 2013;15:492–499.
(10) Carapeti EA, Kamm MA, Phillips RKS. Topical phenylephrine increases anal sphincter resting pressure. Br J Surg. 1999;86:267–270.
(11) Carapeti EA, Kamm MA, Phillips RKS. Randomized controlled trial of topical phenylephrine in the treatment of faecal incontinence. Br J Surg. 2000;87:38–42.
(12) Cheetham MJ, Kamm MA, Phillips RK. Topical phenylephrine increases anal canal resting pressure in patients with faecal incontinence. Gut.. 2001;48:356–359.
(13) Badvie S, Andreyev HJN. Topical phenylephrine in the treatment of radiation-induced faecal incontinence. Clin Oncol. 2005;17:122–126.
(14) Park JS, Kang SB, Kim DW, Namgung HW, Kim HL. The efficacy and adverse effects of topical phenylephrine for anal incontinence after low anterior resection in patients with rectal cancer. Int J Colorectal Dis. 2007;22:1319–1324.
(15) Speakman CT, Hoyle CH, Kamm MA, Henry MM, Nicholls RJ, Burnstock G. Adrenergic control of the internal anal sphincter is abnormal in patients with idiopathic faecal incontinence. Br J Surg. 1990;77:1342–1344.
(16) O'Kelly T, Brading A, Mortensen N. In vitro response of the human anal canal longitudinal muscle layer to cholinergic and adrenergic stimulation: evidence of sphincter specialization. Br J Surg. 1993;80:1337–1341.
(17) Nisar PJ, Gruss H-J, Bush D, Barras N, Acheson AG, Scholefield JH. Intra-anal and rectal application of L-erythro methoxamine gel increases anal resting pressure in healthy volunteers. Br J Surg. 2005;92:1539–1545.
(18) Nisar PJ, Gruss H-J, Bush D, Acheson AG, Scholefield JH. Intra-anal application of L-erythro methoxamine gel increases anal resting pressure in patients with incontinence. Br J Surg. 2007;94:1155–1161.
(19) Jones OM, Thompson JM, Brading AF, Mortensen NJ. L-Erythro-methoxamine is more potent than phenylephrine in effecting contraction of internal anal sphincter in vitro. Br J Surg. 2003;90:872–876.
(20) Mills K, Hausman N, Chess-Williams R. Characterization of the alpha1-adrenoceptor subtype mediating contractions of the pig internal anal sphincter. Br J Pharmacol. 2008;155:110–117.
(21) Rayment SJ, Eames T, Simpson JA, Dashwood MR, Henry Y, Gruss H, et al. Investigation of the distribution and function of alpha-adrenoceptors in the sheep isolated internal anal sphincter. Br J Pharmacol. 2010;160:1727–1740.
(22) Lorenzi B, Brading AF, Martellucci J, Cetta F, Mortensen NJ. Short-term effects of neoadjuvant chemoradiotherapy on internal anal sphincter function: a human in vitro study. Dis Colon Rectum.. 2012;55:465–472.
(23) Melville JL, Fan MY, Newton K, Fenner D. Faecal incontinence in US women: a population-based study. Am J Obstet Gynecol. 2005;193:2071–2076.
(24) Pretlove SJ, Radley S, Toozs-Hobson PM, Thompson PJ, Coomarasamy A, Khan KS. Prevalence of anal incontinence according to age and gender: a systematic review and meta-regression analysis. Int Urogynecol J Pelvic Floor Dysfunct. 2006;17:407–417.
(25) Regadas FS, Batista LK, Albuquerque JL, Capaz FR. Pharmacological study of the internal and sphincter in patients with chronic anal fissure. Br J Surg. 1993;80:799–801.
(26) Rudner XL, Berkowitz DE, Booth JV, Funk BL, Cozart KL, D'Amico EB, et al. Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. Circulation.. 1999;100:2336–2343.
(27) Burt RP, Chapple CR, Marshall I. Evidence for a functional alpha 1A- (alpha 1C-) adrenoceptor mediating contraction of the rat epididymal vas deferens and an alpha 1B-adrenoceptor mediating contraction of the rat spleen. Br J Pharmacol. 1995;115:467–475.
(28) Goetz AS, King HK, Ward SD, True TA, Rimele TJ, Saussy Jr DL. BMY 7378 is a selective antagonist of the D subtype of alpha 1-adrenoceptors. Eur J Pharmacol. 1995;272:R5–R6.
(29) Kenny BA, Chalmers DH, Philpott PC, Naylor AM. Characterization of an alpha 1D- adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br J Pharmacol. 1995;115:981–986.
(30) Williams TJ, Blue DR, Daniels DV, et al. In vitro alpha1-adrenoceptor pharmacology of Ro 70-0004 and RS-100329, novel alpha1A-adrenoceptor selective antagonists. Br J Pharmacol. 1999;127:252–258.
(31) Israilova M, Tanaka T, Suzuki F, Morishima S, Muramatsu I. Pharmacological characterization and cross talk of alpha1a- and alpha1b-adrenoceptors coexpressed in human embryonic kidney 293 cells. J Pharmacol Exp Ther. 2004;309:259–266.
(32) Kobayashi M, Shimizu T. Pharmacological and clinical profile of silodosin (UREF Cap. 2 mg, 4 mg). Folia Pharmacol Jpn. 2006;128:259–268.
(33) Sathi ZS, Anisuzzaman AS, Morishima S, Suzuki F, Tanaka T, Yoshiki H, Muramatsu I. Different affinities of native alpha1B-adrenoceptors for ketanserin between intact tissue segments and membrane preparations. Eur J Pharmacol. 2008;584:222–228.
(34) Nasu K, Moriyama N, Fukasawa R, Tsujimoto G, Tanaka T, Yano J, Kawabe K. Quantification and distribution of alpha1-adrenoceptor subtype mRNAs in human proximal urethra. Br J Pharmacol. 1998;123:1289–1293.