Characterizations of the $\alpha_1$-adrenoceptor subtypes mediating contractions of the human internal anal sphincter

Hiroyuki Owaki a, *, Sotaro Sadahiro c, Miyako Takaki a, b

a Department of Physiology II, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Nara 634-8521, Japan
b Department of Molecular Pathology, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Nara 634-8521, Japan
c Department of Surgery, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa, 259-1193, Japan

Article history:
Received 1 December 2014
Received in revised form
17 February 2015
Accepted 26 February 2015
Available online 6 March 2015

Keywords:
Human internal anal sphincter
$\alpha_1$-adrenoceptor
Fecal incontinence
Age-related response change
Comparison with inferior mesenteric artery
As a predictor of systemic arterial pressure

Abstract

Human internal anal sphincter (IAS) is contracted by $\alpha_1$-adrenoceptor stimulation and thus $\alpha_1$-adrenoceptor agonists may be useful in treating fecal incontinence. This study characterizes the contribution of $\alpha_1$-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 $\alpha_1$-adrenoceptor antagonists. Immunohistochemistry and gene expression studies were performed in the same samples. The mean pEC50 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 pEC50 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 $\alpha_1$-adrenoceptor antagonists. Each $pK_a$ value of silodosin, BMV-7378 and prazosin was 9.36 ± 0.53, 7.28 ± 0.20 and 8.89 ± 0.12, respectively. These $pK_a$ values and gene expression studies indicated that $\alpha_{1A}$-adrenoceptor subtypes predominantly contributed to human IAS contraction.

© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of Japanese Pharmacological Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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). Alpha1-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 $\alpha_{1A}$-adrenoceptor agonist, L-erythro-methoxamine was characterized with higher $\alpha_{1A}$-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 $\alpha_1$-adrenoceptor agonist without elevating blood pressure.

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

* Corresponding author. Departments of Physiology II, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Nara 634-8521, Japan. Tel.: +81 744 23 8820; fax: +81 744 23 4696.
E-mail address: hiroyuki-owaki@naramed-u.ac.jp (H. Owaki).
Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.02.016
1347-8613/© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of Japanese Pharmacological Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The contribution of $\alpha_1$-adrenoceptor subtypes have been studied in IAS smooth muscle of pig (19, 20) and sheep (21); $\alpha_{1A}$-adrenoceptor contracted it in the pig (20), whereas the $\alpha_{1A}$- and $\alpha_{2D}$-adrenoceptors contracted it in the sheep (21). However, there is no reliable information concerning $\alpha_1$-adrenoceptor subtypes in human IAS. The first aim of the current study was to characterize the contribution of $\alpha_1$-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% CO₂ 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% CO₂ in oxygen. One end of each strip was connected to a force-displacement transducer (SB-1T, NHIRO KOHDEN, Tokyo, Japan), and changes in muscle tension were measured and recorded on a pen-writing oscillograph (Rectigraph 85, NEC-Sanei, Tokyo, Japan). The preinations were allowed to equilibrate with 10 nM 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 $\alpha_1$-adrenoceptor antagonists. The following antagonists were used: the selective $\alpha_{1A}$-adrenoceptor antagonist, silodosin (3 nM); the selective $\alpha_{1D}$-adrenoceptor antagonist, BMY-7378 (3 µM) and the non-subtype-selective $\alpha_1$-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 (pEC₀ values) were calculated by non-linear regression fitting to sigmoidal concentration–response curves (variable slope) by GraphPad Prisma™ software version 4 (GraphPad Software Inc, San Diego, USA). Individual affinity estimates (apparent pKₐ values) were determined from the equation: [pKₐ = log (EC₀ with antagonist/EC₀ without antagonist)]. 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, which was difficult to obtain human tissues for laboratory studies, and could not use more concentrations of antagonists for calculation to pA₂ 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 $\alpha_{1A}$-adrenoceptor (ID: HA073254), $\alpha_{1B}$-adrenoceptor (ID: HA032618), and $\alpha_{1D}$-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 $\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-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 $\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptors and secondary antibody (Arcis Antibodies, Hidenhausen, 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$_2$PO$_4$ 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 $\alpha_{1}$-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 $\alpha_{1A}$- and $\alpha_{1D}$-adrenoceptors, but was weakly stained by $\alpha_{1B}$-adrenoceptor (Fig. 2). Moreover, peripheral nerve bundles were stained by the $\alpha_{1A}$-adrenoceptor and blood vessel intimal smooth muscle was stained by the $\alpha_{1D}$-adrenoceptor.

#### 3.4. Gene expression levels of $\alpha_{1}$-adrenoceptor subtypes in IAS

The mRNA expressions of $\alpha_{1}$-adrenoceptor subtypes were confirmed by quantitative PCR (Fig. 3A). The gene expression of $\alpha_{1A}$- and $\alpha_{1D}$-adrenoceptors in IAS was not different but that of $\alpha_{1B}$-adrenoceptor was negligible ($P < 0.05$). In addition, each gene expression of the $\alpha_{1A}$-adrenoceptor and $\alpha_{1D}$-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).

### 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 $\alpha_{1}$-adrenoceptor stimulation. To the best of our knowledge, no reliable
information about α1-adrenoceptor subtypes in human IAS has been reported; however, topical anal treatment with α1-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 α1A-adrenoceptor agonist. Previous reports using noradrenaline and phenylephrine in IAS of healthy volunteers showed similar contractions, although there was no information about α1-adrenoceptor subtypes (15, 16, 25).

Marked individual differences in pEC50 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 pEC50 value of IAS for phenylephrine than that of IMA was observed. This result suggested the possibility of differentiating effects of α1-adrenoceptor agonist, phenylephrine on IAS compared with that on IMA according to patient conditions.

The pEC50 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 1
Patient information and each of EC50 value for phenylephrine in human internal anal sphincter (IAS) and inferior mesenteric artery (IMA) smooth muscles.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gender</th>
<th>Age (y)</th>
<th>IAS pEC50</th>
<th>IAS maximum response (mN)</th>
<th>IMA pEC50</th>
<th>IMA maximum response (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elderly group (≥70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>70</td>
<td>5.85</td>
<td>16.64</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>73</td>
<td>5.55</td>
<td>8.65</td>
<td>5.03</td>
<td>20.99</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>70</td>
<td>6.12</td>
<td>3.88</td>
<td>5.52</td>
<td>10.81</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>80</td>
<td>5.86</td>
<td>7.11</td>
<td>6.05</td>
<td>12.01</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>72</td>
<td>6.66</td>
<td>16.27</td>
<td>5.79</td>
<td>30.79</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>87</td>
<td>6.37</td>
<td>33.08</td>
<td>5.56</td>
<td>20.45</td>
</tr>
<tr>
<td>Younger group (&lt;70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>60</td>
<td>6.23</td>
<td>3.55</td>
<td>5.88</td>
<td>5.63</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>57</td>
<td>6.38</td>
<td>10.64</td>
<td>5.23</td>
<td>25.06</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>69</td>
<td>6.77</td>
<td>8.87</td>
<td>5.56</td>
<td>13.12</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>36</td>
<td>6.58</td>
<td>5.37</td>
<td>5.91</td>
<td>15.03</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>60</td>
<td>6.96</td>
<td>27.44</td>
<td>5.44</td>
<td>24.97</td>
</tr>
</tbody>
</table>

The upper part; All patients were divided into two groups by age. Data show the mean pEC50 of 3–6 smooth muscle strips. The lower part; Data show the mean of age and mean ± SEM of pEC50. Student’s paired t-test. *P < 0.05 vs IMA pEC50 of total case. †P < 0.05 vs IAS pEC50 of the Elderly group. ‡P < 0.05 vs IMA pEC50 of the younger group.

### Table 2
Affinity values of various antagonists of α1-adrenoceptor in human internal anal sphincter (IAS) smooth muscles.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Published pKB (pA2) values and SEM of α1-adrenoceptor subtypes</th>
<th>Mean pKB values and SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α1A</td>
<td>α1B</td>
</tr>
<tr>
<td>Silodosin</td>
<td>9.86 ± 0.32</td>
<td>7.52 ± 0.30</td>
</tr>
<tr>
<td>BMY-7378</td>
<td>6.30 ± 0.15</td>
<td>6.93 ± 0.25</td>
</tr>
<tr>
<td>Prazosin</td>
<td>9.20 ± 0.29</td>
<td>9.60 ± 0.14</td>
</tr>
</tbody>
</table>

Published pKB (pA2) values obtained from functional and binding studies (27–33). Mean pKB values with SEM for antagonists obtained using phenylephrine as the agonist in human IAS (n = 8–9).

![Human IAS](image)

Fig. 2. Immunohistochemical analysis by staining with anti-α1A-, anti-α1B- and anti-α1D-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 α1A- and α1D-adrenoceptors, but was weakly stained by α1B-adrenoceptor. Peripheral nerve bundles were stained by the α1A-adrenoceptor (an arrow head in A) and blood vessel intimal smooth muscle was stained by the α1D-adrenoceptor (an arrow in C). Scale bar = 100 μm.
elderly, this information will be relevant to a specific subgroup of patients.

Each gene expression for the $\alpha_{1A}$-adrenoceptor and $\alpha_{1D}$-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 $\alpha_{1A}$-adrenoceptor and $\alpha_{1D}$-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 $\alpha_{1}$-adrenoceptor subtypes in human IAS

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

The pKB value of silodosin was ranked between each published pKB value of $\alpha_{1A}$-adrenoceptor and $\alpha_{1D}$-adrenoceptor. In the pKB value of BMY-7378 is 30-fold (1.5 log units) below its affinity at $\alpha_{1D}$-adrenoceptors in IAS (Table 2). On the other hand, the intermediate affinity obtained for prazosin are mediated via the $\alpha_{1A}$-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 $\alpha_{1A}$-adrenoceptor contribute to contraction of human IAS. This possibility is supported by previous study showing that in the human urethra, the $\alpha_{1}$-adrenoceptor subtype $\alpha_{1A}$ have been reported to contribute to smooth muscle contraction (34).

The IAS contraction is reported to be mediated by $\alpha_{1A}$- and $\alpha_{1D}$-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 $\alpha_{1A}$-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 $\alpha_{1A}$-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 $\alpha_{1A}$-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 $\alpha_{1}$-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 α1-adrenoceptor agonist requires a strategy to minimize the risk of elevated blood pressure among elderly patients.

Conflicts of interest

There is no conflict of interest in this study.

Acknowledgements

The authors thank K. Horie, S. Tazawa, H. Shiohara, H. Takeda for their helpful suggestions, as well as T. Homma, Y. Hotei, and I. Tsuchiya for arranging the sample collection and for supporting the data analysis.

References