The role of nitric oxide (NO) in the human internal anal sphincter

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Introduction

The internal anal sphincter (IAS) is a specialized structure of the distal part of the anal canal. The IAS is a distinct ring of muscle, and it normally functions as one component of the anorectal reflex for defecation, playing an important role in preventing incontinence by maintaining contraction reaction.1 At rest, the major part of the pressure in the anal canal is contributed by the IAS. During distension of the rectum, the IAS undergoes relaxation and this reflex is locally mediated by the enteric nervous system. However, no convincing light has yet been shed on the neurological control of the IAS in humans. During the past decade, with the advances in pharmacology, electrophysiology, and immunohistochemistry, it has been suggested that the nonadrenergic noncholinergic (NANC) nervous system, in addition to the two classic adrenergic and cholinergic components, may play an important role in the regulation of gastrointestinal motility.2–5 Recent pathological studies have shown that nitric oxide (NO) is an inhibitory neurotransmitter in the human gut.6–10 NO synthase has been localized by histochemical processes in the normal lower esophageal sphincter, pyloric sphincter, and colon.11–13 In the present study, we investigated the effects of NO on normal human IAS obtained from patients who underwent rectal amputation for low rectal cancers. The in-vitro effects of NO were assessed, using a mechanographic technique.

Purpose. Nitric oxide (NO) has recently been shown to be a neurotransmitter in nonadrenergic noncholinergic (NANC) inhibitory nerves in the human gut. To clarify the physiological significance of NO in the human internal anal sphincter (IAS), we investigated enteric nervous responses in normal IAS muscle strips above the dentate line, obtained from patients with rectal cancer.

Methods. Normal IAS muscle strips above the dentate line, obtained from ten patients who underwent rectal amputation for low rectal cancers were used. The subjects consisted of eight men and two women, aged from 46–72 years (mean age, 54.2 years). A mechanographic technique was used to evaluate in-vitro IAS muscle responses to electrical field stimulation (EFS) of adrenergic and cholinergic nerves before and after treatment with various autonomic nerve blockers, Nω-nitro-l-arginine (l-NNA) and l-arginine.

Results. Excitatory nerves were mainly involved in the regulation of enteric nerve responses to EFS in the baseline condition of the study, and NANC inhibitory nerves acted on the normal IAS. l-NNA concentration-dependently inhibited the relaxation in response to EFS in the human IAS, and this inhibitory effect in the IAS was reversed by l-arginine. Conclusions. These findings suggest that NANC inhibitory nerves play important roles in regulating relaxation of the human IAS, and that NO plays an important role as a neurotransmitter in NANC inhibitory nerves of the human IAS.

Key words: internal anal sphincter, nonadrenergic noncholinergic inhibitory nerve, neurotransmitter, nitric oxide, motility

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Mucosal tissues were removed from the IAS tissues, and muscle strips approximately 1.0 cm in length and 0.3 cm in width, were prepared in the direction of the circular muscles. The materials were placed in a 10-ml organ bath containing Krebs solution (NaCl, 6.9 g/l; KCl, 0.35 g/l; MgSO₄·7H₂O, 6.29 g/l; KH₂PO₄, 0.16 g/l; CaCl₂·2H₂O, 0.37 g/l; glucose, 2.0 g/l; NaHCO₃, 2.1 g/l; Kantonkagaku, Tokyo, Japan) at 37°C, and gassed with 95% O₂ and 5% CO₂. The movement of the muscle strips was recorded with a pen recorder (model R-10; Rica Denki, Tokyo, Japan) through an isotonic transducer (model ME-4012; ME Commercial, Tokyo, Japan) given a 1-g load. The enteric nerves of the muscle strips were stimulated electrically with a clip (Selfin clip; ME Commercial) that was held at both ends and connected to a stimulator (model ME-6052; ME Commercial). A low frequency of 5 Hz, which is suitable for the stimulation of nerves, with a duration of 0.5 ms, voltage of 50 V, and stimulation time of 30 s was employed. The stimulation was given according to the square-wave repetitive method. The responses to electrical field stimulation (EFS) were investigated in the presence and absence of various autonomic nerve blockers, N⁶-nitro-L-arginine (L-NNA), and L-arginine. IAS movements were recorded once the muscle strips had become stabilized, after 1 h. The following drug preparations were used: atropine sulfate 1 × 10⁻⁷ g/ml (Sigma, St. Louis, MO, USA); phenoxybenzamine, 5 × 10⁻⁶ g/ml (Sigma); tetrodotoxin, 5 × 10⁻⁷ g/ml (Sankyo, Tokyo, Japan); L-NNA (1 × 10⁻⁸, 1 × 10⁻⁷, and 1 × 10⁻⁶ g/ml; Sigma); and L-arginine (1 × 10⁻⁸, 1 × 10⁻⁷, 1 × 10⁻⁶ g/ml; Nakarai, Tokyo, Japan).

Statistical analysis

As the observational unit, we considered a preparation, not a patient, because between two and three muscle strips per patient were used. The χ² test (two-tailed) was used. A P value of less than 0.05 was regarded as significant.

Results

Experiment 1

To determine whether NANC excitatory and inhibitory nerves were present in the IAS, the responses to EFS before and after blockade of the adrenergic and cholinergic nerves were studied.

The responses to EFS before blockade of the adrenergic and cholinergic nerves are shown in Table 1, section a. Figure 1A illustrates the results of a typical experiment. A contraction reaction and a relaxation reaction were seen, respectively, in 69.2% and 30.8% of the IAS muscle strips. In addition, there were significant differences between the frequency of contraction responses and those of relaxation responses (P < 0.01). The responses to EFS after blockade of the adrenergic and cholinergic nerves are shown in Table 1, section b. Figure 1B illustrates the results of a typical experiment. A contraction reaction and a relaxation reaction were seen, respectively, in 7.7% and 92.3% of the normal IAS muscle strips. The muscle strips in the normal IAS demonstrated relaxation reactions rather than contraction reactions by EFS. In addition, there were significant differences between the frequency of contraction responses and those of relaxation responses (P < 0.001). The frequency of relaxation responses after the blocking of the adrenergic and cholinergic nerves was significantly greater than that before blocking (P < 0.001). Responses to EFS following administration of tetrodotoxin are shown in Table 1, section c. It was thus uncertain whether EFS responses reacted via the nerves or by a direct effect on the smooth muscle. To clarify this point, a study was made of the effects of EFS after blocking the total enteric nervous systems with tetrodotoxin following these experiments. Figure 1C illustrates the results of one typical experiment. Tetrodotoxin abolished the EFS responses in the normal IAS muscle strips (Table 1, second c).

These results indicated that NANC excitatory and inhibitory nerves were involved in the regulation of enteric nerves in the muscle strips of IAS.

Experiment 2

The effect of L-NNA (an inhibitor of NO biosynthesis) on EFS was studied after the blocking of both the adrenergic and cholinergic nerves.

Table 1. Experiment 1. To determine whether NANC inhibitory nerves were present in the human IAS, the responses to EFS before and after blockade of the adrenergic and cholinergic nerves, and after the addition of tetrodotoxin, were studied.

<table>
<thead>
<tr>
<th></th>
<th>No reaction</th>
<th>Contraction</th>
<th>Relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0% (0/26)</td>
<td>69.2% (18/26)</td>
<td>30.8% (8/26)</td>
</tr>
<tr>
<td>b</td>
<td>No reaction</td>
<td>7.7% (2/26)</td>
<td>** 92.3% (24/26)</td>
</tr>
<tr>
<td>c</td>
<td>No reaction</td>
<td>100% (26/26)</td>
<td>0% (0/26)</td>
</tr>
</tbody>
</table>

* P < 0.01; ** P < 0.001

NANC, nonadrenergic noncholinergic; IAS, internal anal sphincter; EFS, electrical field stimulation

a. Response to EFS before blockade of the adrenergic and cholinergic nerves
b. Response to EFS after blockade of the adrenergic and cholinergic nerves
c. Response to EFS following administration of tetrodotoxin after blockade of the adrenergic and cholinergic nerves