Evaluation of adaptive relaxation of the rat stomach using an orally inserted balloon instead of surgical intervention by demonstrating the effects of capsaicin and \( \text{N}^\omega \)-nitro-L-arginine methylester

Masayuki UCHIDA1 and Kimiko SHIMIZU1

1Food Science Institute, Meiji Co., Ltd. Japan

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Abstract

Gastric functions such as adaptive relaxation have usually been monitored in rats using a surgically inserted barostat’s balloon. However, surgery causes physiological damage to the rat stomach. This study is an investigation of adaptive relaxation of the rat stomach using a slightly modified balloon, which is introduced into the stomach through the mouth of anesthetized rats without the need for balloon surgery, attached to a brostat. In this case, the balloon was placed between the fore-stomach and the fundus, but towards the fore-stomach. The balloon volume increased gradually just after an increment in the balloon pressure, and reached a plateau within 1 min. This increased volume just after the increment of the balloon pressure was defined as adaptive relaxation. Adaptive relaxation increased with pressure increases in a pressure dependent manner. Pre-treatment with \( \text{N}^\omega \)-nitro-L-arginine methylester (30 mg/kg, i.v.) caused this adaptive relaxation to be significantly inhibited as compared with the control. On the contrary, adaptive relaxation was significantly enhanced by pre-treatment with capsaicin (0.5 mg/kg, p.o.). These findings show that this method is both useful for investigating the physiology of adaptive relaxation of the stomach without surgery and to show that nitric oxide plays an important role in the adaptive relaxation of the stomach as reported previously.

Key words: adaptive relaxation, stomach of rat, capsaicin, L-NAME, NO

Introduction

In clinical studies, a barostat has been used for the investigation of gastric functions, such as changes in gastric pressure or accommodation (adaptive relaxation) of the stomach. On the other hand, in basic studies using experimental animals, there are a number of investigations that have been made...
of the changes in intra-gastric pressure by either surgically inserting the pressure transducer into the rat stomach (Desai et al., 1991a, Desai et al., 1991b, Hayakawa et al., 1999), by connecting a pressure transducer to the isolated guinea pig stomach (Takahashi et al., 1997a, 1997b) or by inserting the balloon into the rat stomach via an incision in the fundus (Ozaki et al., 2002). However, it is easy to understand that the surgical procedures mentioned above affect normal gastric physiological function in an in vivo study, and that in vitro studies do not necessarily reflect the in vivo state. It was our intention in this study to establish a method that would enable us to monitor gastric pressure using a balloon and a barostat without the surgical intervention that has been done in clinical studies.

Adaptive relaxation has been known to occur when food passes down the pharynx and the esophagus or during the early stages of vomiting. Zhao et al. (2010) reported that functional vomiting had significant postprandial gastric dysrhythmia and impaired gastric accommodation. It has also been shown that non-adrenergic and non-cholinergic (NANC) mediators, such as adenosine triphosphate, nitric oxide (NO) and vasoactive intestinal peptide, are involved in gastric relaxation (Currò et al., 2008). Desai et al. (1991a, b) have reported that NO mediated adaptive relaxation induced by stimulation of the vagus nerve in the isolated stomach of the guinea pig. NO has been known to relax the smooth muscle and to be synthesized by NO synthase from L-arginine. \( N^\omega \)-Nitro-L-arginine methyl ester (L-NAME) inhibits the NO synthase non-selectively. On the other hand, capsaicin has been known to activate capsaicin sensitive afferent nerve and release NO or calcitonin gene-related peptide (CGRP) (Brzozowski et al., 2005). Thus, in the present study we have evaluated the effect of capsaicin and L-NAME on adaptive relaxation to validate our improved method.

**Materials and Methods**

The following animal studies were performed in accordance with the *Guiding Principles for the Care and Use of Laboratory Animals* approved by Meiji Co., Ltd.

**Animals**

Male Sprague-Dawley rats (230–280 g) were purchased from SLC (Shizuoka, Japan) and kept for 1 week in a room where the temperature and humidity were kept at 21 ± 2°C and 55 ± 15%, respectively. The animals were fasted in mesh cages for 18 h before each experiment in order to prevent coprophagy, but were allowed free access to drinking water during this period.

**Gastric barostat study**

Rats were anesthetized with urethane (1.2 g/kg, i.p.). In this study, a slightly improved balloon was used. A pair of polyvinyl tubes attached to a polyethylene bag (maximum volume 5 mL; 3 cm maximum diameter) was introduced through the mouth into the stomach as shown in Fig. 1. Five mL of air was injected into the balloon from one of the balloon tubes with the other side balloon tube closed to allow placement of the balloon within the stomach, after which the balloon tubes were immediately opened to the air. After a 10 min recovery period, the tubes of the balloon were connected to the barostat (Barostat Distender IIR, G&J Electronics, Toronto, Canada) as shown in Fig. 1.

The pressure inside the balloon was increased stepwise from 1 through 2, 4 and 8 mm Hg, at 1
min intervals. The volume of the balloon increased sharply with each change in pressure as shown in Fig. 2. The balloon volume increased gradually just after the change of pressure and reached a plateau after about 1 min following the change of pressure. At that stage, the increased volume was defined as adaptive relaxation (accommodation).
Effect of capsaicin on the adaptive relaxation

Capsaicin was dissolved in 5% ethanol and administered orally to the rat at a dose of 0.5 mg/kg (2.5 mL/kg body weight) 30 min before the barostat study. Rats were anesthetized with urethane just before the barostat study was performed. In the control rats, 5% ethanol was administered instead of capsaicin.

Effect of L-NAME on the adaptive relaxation

L-NAME was dissolved in distilled water for injection and administered intravenously to the rat at a dose of 30 mg/kg (0.1 mL/100 g body weight) 10 min before the barostat study. Rats were anesthetized with urethane just before the barostat study was performed. In the control rats, distilled water for injection was administered intravenously instead of L-NAME.

Data analysis

All results are presented as the mean ± S.E.M. Statistical analyses were performed by using Stat View, Version 5.0.0.0 (SAS Institute Inc., USA), and P values <0.05 (Student’s t-test) were considered to be statistically significant.

Agents

Capsaicin and L-NAME were purchased from Sigma (Tokyo, Japan) and Wako Pure Chemical (Tokyo Japan), respectively. Distilled water for injection was obtained from Ohtsuka Seiyaku Industry (Tokushima, Japan).

Results

Gastric barostat study

Adaptive relaxation increased in a pressure-dependent manner (Fig. 3).
Effect of capsaicin on the adaptive relaxation

In the control rats, the adaptive relaxation increased in a pressure-dependent manner (Fig. 4). Adaptive relaxation was significantly enhanced by pre-treatment with capsaicin at 8 mmHg \((P<0.05)\).

Effect of \(\text{L-NAME}\) on the accommodation

In the control rats, adaptive relaxation increased in a pressure-dependent manner (Fig. 5). Adaptive relaxation was significantly inhibited by pre-treatment with \(\text{L-NAME}\) at 8 mmHg \((P<0.01)\).

Discussion

In this study, we observed adaptive relaxation of the stomach by increasing the intragastric pressure. This adaptive relaxation was significantly enhanced by pre-treatment with capsaicin and significantly inhibited by pre-treatment with \(\text{L-NAME}\).
The basic research studies performed in this field using a barostat have generally used surgical procedures \textit{in vivo}. In other words, the balloon was inserted into the stomach for monitoring the adaptive relaxation of the stomach through an incision in both the abdominal wall and the wall of the corpus or antrum of the stomach. But these operations are invasive and possibly nonphysiological because of the undesirable damage to the rat stomach. Therefore, these methods were considered to be unsuitable for investigating the normal physiological function of the stomach. In this study, we easily introduced the balloon from the mouth to the stomach. We found that after each pressure increment within the balloon, there was a simultaneous increase in the volume of the balloon. After each increment of pressure, the volume of the balloon increased gradually and reached a plateau within 1 min. The increased volume after the change of the balloon pressure was defined as adaptive relaxation. Adaptive relaxation increased with each increment in the balloon pressure. Hayakawa \textit{et al.} (1999) reported the adaptive relaxation of the stomach in an \textit{in vitro} study of the guinea pig and found that the stomach volume increased in a pressure-dependent manner. These results are in accord with our present results. In general, adaptive relaxation was regarded as the rapid increase of the balloon volume which occurred after an increase in the balloon pressure in the isolated guinea pig stomach (Desai \textit{et al.} 1991a). However, with our method, a rapid increase in the balloon volume was not observed. This is in contrast to the reports by Desai \textit{et al.} (1991a, b) and Hayakawa \textit{et al.} (1999), who tested adaptive relaxation in preparations that had been treated with atropine and guanethidine to negate the effects of both cholinergic and adrenergic nerves in the isolated guinea pig stomach. In our experiments, we did not use these drugs, which might explain the differences we found in the adaptive relaxation volume. Treatment with atropine and guanethidine may even reduce the results obtained using our method.

From the results of our study, it became possible to monitor the change of the volume of the balloon and adaptive relaxation by stepwise increments of the balloon pressure without surgical injury. Therefore, this method was found to be suitable for monitoring the adaptive relaxation of the stomach without surgical intervention.

Adaptive relaxation has been known to be mediated by capsaicin sensitive afferent nerves. Lee \textit{et al.} (2004) reported that the acute administration of capsaicin decreased proximal gastric tone and inhibited phasic contractility of the human proximal stomach. Tonini \textit{et al.} (2000) also reported the role of NO- and VIP-containing neurones in human gastric fundus strip relaxations. NO has been supposed to be the final mediator causing relaxation of the stomach. Desai \textit{et al.} (1991a) reported that adaptive relaxation in the isolated stomach of the guinea pig is mediated by a NANC neurotransmitter substance that is indistinguishable from NO derived from L-arginine by NO synthase. NO has been known to relax smooth muscle by activating guanylate cyclase. Therefore, we tested the effects of agents involved in NO generation on the adaptive relaxation to validate our method.

Capsaicin has been known to activate afferent nerves and release NO and CGRP (Lefebvre \textit{et al.}, 1991). In this study, treatment with capsaicin significantly enhanced the adaptive relaxation which resulted from increases in the balloon volume as compare with the control. Lee \textit{et al.} (2004) reported that the acute administration of capsaicin decreased proximal gastric tone and inhibited phasic contractility of the human proximal stomach. Desai \textit{et al.} (1991b) reported that nitricergic nerves mediate vagally induced relaxation in the isolated stomach of the guinea pig. These findings support our present results with capsaicin and show that capsaicin enhances the adaptive relaxation of the stomach through NO or CGRP.
L-NAME has been known to inhibit NO synthase non-selectively (Bishop-Bailey et al., 1997). Therefore, we tested the effect of L-NAME on adaptive relaxation of the stomach with our experimental procedure. We found that L-NAME treatment significantly inhibited adaptive relaxation as a result of increments in the balloon pressure as compared with the control. This finding shows that the adaptive relaxation of the stomach is induced through the generation of NO. Janssen et al. (2008, 2010) showed that L-NAME significantly increased intragastric pressure during stomach distension, indicating decreased gastric accommodation. These reports are in accord with our present result with L-NAME. The present results and the published reports support the concept that adaptive relaxation is mediated through actions of capsaicin sensitive afferent nerves and NO.

With the present experimental system, the effect of the central nervous system is uncertain as the rats were anesthetized with urethane. However, central mediation could still be demonstrated in experiments investigating gastric acid secretion in anesthetized animals (Yano et al., 1990; Watanabe et al., 2000). Therefore, the effect of the central nervous system on gastric adaptive relaxation should still be able to be evaluated at least in part, although the rats were not in a fully conscious state.

In conclusion, one of the merits of this evaluation system is that there is no surgical damage. The effect of drugs affecting the central nervous system could be evaluated, although the rats were not in a fully conscious state. Moreover, it was confirmed at least in part, that capsaicin induced adaptive relaxation of the stomach occurs through the generation of NO.

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