



Original

Method for detecting hemodynamic alterations following a single gavage in rats

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Abstract: It is known that administering a gavage to rodents evokes a cardiac reflex, due to gastrointestinal stimulation. Consequently, it is difficult to evaluate changes in hemodynamics after a single oral dose of a pungent or astringent, which alters the circulation by increasing sympathetic activity. In the present study, we developed a method for administering a gavage without significantly affecting hemodynamics measurements. We marked a gastric tube at 10 cm from the tip, to mark the distance from the oral cavity to the stomach body of Wistar male rats. Rats were intubated under urethane anesthesia. After 10–15 min of stabilization, we measured the mean blood pressure (MBP), heart rate (HR), and blood flow (BF) in the cremaster arteriole under two different conditions; condition 1: a pointed gastric tube, room temperature distilled water, and injected at normal speed (approximately 3 ml/min); condition 2: a rounded gastric tube, 37°C distilled water, and injection at 1.0 ml/min. Under condition 1, we observed striking hemodynamic alterations, due to the somatic afferent reflex. In contrast, under condition 2, these hemodynamic changes were nearly eliminated. In addition, we could clearly detect hemodynamic changes in rats after a single gavage treatment of pungent (capsaicin) or astringent (cinnamtannin A2). We observed transient increases in the HR and MBP soon after treatment with capsaicin. Moreover, cremasteric BF was elevated with cinnamtannin A2. These results confirmed the utility of the gavage method developed in this study.

Key words: blood pressure, cremasteric blood flow, gavage administration, heart rate, rat

Introduction

When we eat food that is rich in pungent and/or astringent substances, significant changes occur in our hemodynamics, due to an enhancement in sympathetic nerve activity [1, 2]. The administration of a gavage in rodents also results in marked temporary changes in heart rate (HR) and blood pressure, due to a cardiac reflex in response to gastrointestinal tract stimulation. It is well known that the mechanism underlying these physiological responses involves visceral-sympathetic reflexes [3]. Consequently, it is difficult to evaluate essential changes in hemodynamics following a single gavage admin-

istration of a pungent or astringent substance. There is a need for a measuring system that can minimize the mechanical and thermal stimulations caused by gavage administration. In the present study, we developed a method for delivering an experimental gavage without interfering with measurements of HR, mean blood pressure (MBP), and cremaster arteriole blood flow (BF) in rats. In addition, we confirmed the utility of our method for chemicals that exhibits sympathetic nerve hyperactivity, using typical pungent capsaicin for the confirmation of the measurement method for HP and BF or typical astringent cinnamtannin A2 for BF in cremasteric arteriole.

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Materials and Methods

Animals and diets

This study was approved by the Animal Care and Use Committee of the Shibaura Institute of Technology (Permit Number; AEA19016). All animals received humane care under the guidelines of this institution. Male Wistar rats that weighed 200–250 g were obtained from Saitama Experimental Animal Supply (Tokyo, Japan). The rats were maintained in a room with controlled lighting (12 h light/dark cycles) and regulated temperature (23–25°C). We measured the distance from the oral cavity to the stomach body, and the average was 10 cm (n=5). Rats were fed a certified diet obtained from the Oriental Yeast Co., Ltd., Tokyo, Japan. After the measurement was completed, the rats were euthanized by phlebotomy.

Materials

Urethane, phosphate-buffered saline (PBS), tween 80, ethanol, and capsaicin were purchased from Sigma Chemicals (St. Louis, MO, USA). Cinnamtannin A2, an (–)-epicatechin tetramer, was purchased from Phytolab GmbH & Co., KG (Vestenbergsgreuth, Germany). We used 250-mm polyethylene tubes purchased from HAKKO Co., Ltd., (Nagano, Japan) for the gastric tubes. We marked the gastric tubes at 10 cm from the tip and attached them to a 2.5-ml syringe.

Experimental procedure

Rats were placed under anesthesia with urethane (1 g/kg, subcutaneously). In this experiment, we selected urethane as anesthesia agent, which has the least effect on the cardiovascular system, to be satisfied our purpose for observing changes in circulatory dynamics. In addition, we were unable to use inhalation anesthesia, because to investigate the effects of oral administration in this study. A gastric tube was inserted into the oral cavity and advanced into the stomach body, stopping at the 10-cm mark on the tube. Baseline observations were carried out for 10 to 15 min. Distilled water (4 ml/kg body-weight) was administrated by gavage to rats under two different conditions: condition 1: the gastric tube was pointed, the distilled water was room temperature, and injected at normal speed (approximately 3 ml/min); condition 2: the gastric tube was rounded, the distilled water was 37°C, and the injection was delivered at 1.0 ml/min.

The round tube was made using a fine file. We filed at right angles until the tip of the tube went from transparent to translucent and softened.

HR and MBP measurements

Changes in HR and MBP were determined in rats non-invasively with the tail-cuff method and the BP98A system (Softron, Tokyo Japan; Fig. 1a) [4]. We measured the HR and MBP every 6 min for 60 min after a single gavage administration (Fig. 1b).

BF measurements in the cremaster arteriole

We measured BF in the rat cremaster arteriole according to the method previously reported [4]. In brief, the cremaster muscle was exteriorized and carefully spread out in a dedicated plastic chamber with an optical port for transillumination. The muscle surface was superfused with PBS (pH 7.3–7.4) at 37°C. After a 10–15-min post-surgical equilibration period, we selected a single, unbranched arteriole with a resting inner diameter of 15–20 μm . After the baseline observation, we administered a single gavage and measured the cremasteric arterial BF with a laser Doppler blood flowmeter (Periscan PIM-2, Perimed Co., Ltd., Stockholm, Sweden) for 60 min (Figs. 2a and b).

Alterations in the MBP and HR after gavage administration of a pungent substance

To test alterations in the MBP and HR due to a pungent substance, we used capsaicin. After the baseline observation, we administered a gavage of either vehicle (10% tween 80, 3% ethanol in saline, n=8) or capsaicin (600 $\mu\text{g}/\text{kg}$ body-weight, n=4). We measured the changes in MBP and HR every 6 min for 60 min.

Alterations in cremaster arteriole BF after gavage administration of an astringent substance

To measure alterations in cremaster arteriole BF due to an astringent substance, we used cinnamtannin A2. After the baseline observation, we administered a single gavage of either vehicle (3% tween 80 in distilled water, n=8) or cinnamtannin A2 (10 $\mu\text{g}/\text{kg}$ body-weight, n=8). We measured the change in cremasteric BF for 60 min.

Data analysis and statistical methods

We performed a 10-min preliminary measurement prior to administration of the test compound and used the average of the data obtained as the reference value. Each value represents the difference between the standard value and the value after administration of the reagent. Typical data for each group (Figs. 1 and 2) or the means and standard deviations (Figs. 3 and 4) were expressed. Statistical analyses were performed with the Kruskal-Wallis test, followed by post hoc comparisons between experimental groups with non-parametric Wilcoxon and Mann-Whitney U tests after Bonferroni cor-

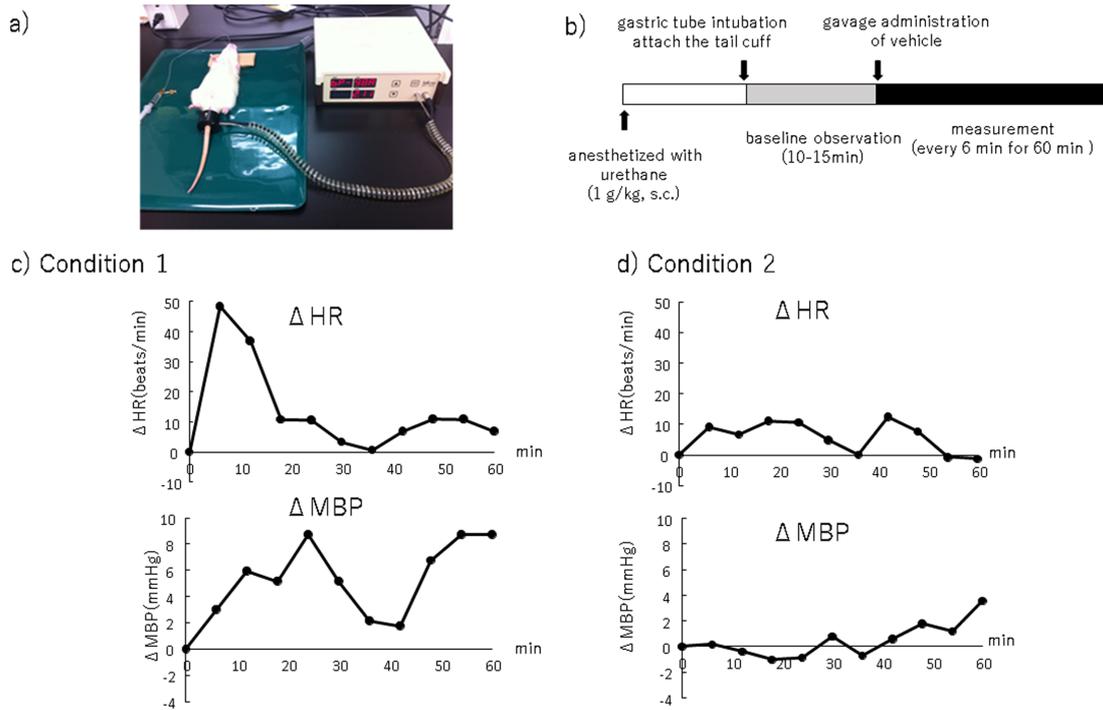


Fig. 1. Measurements of heart rate (HR) and mean blood pressure (MBP) under two different gavage conditions. Condition 1: a pointed gastric tube, room-temperature distilled water, injected at normal speed (approximately 3 ml/min); condition 2: a rounded gastric tube, 37°C distilled water, and slow injection (1.0 ml/min). (a) Photograph of the equipment used in the experiment (BP98A system); (b) diagram of the experimental procedure; s.c.: subcutaneous; (c) representative measurements of HR and MBP in an anesthetized rat under condition 1; (d) representative measurements of HR and MBP in an anesthetized rat under condition 2.

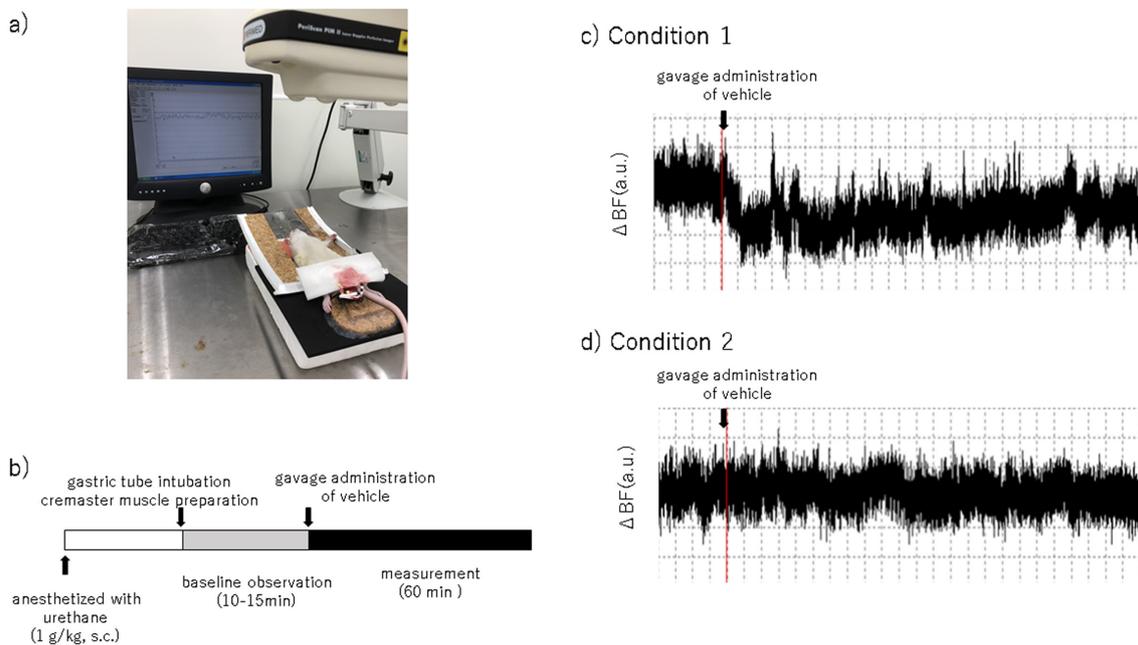


Fig. 2. Measurements of blood flow (BF) in the cremaster muscle arteriole under two different gavage conditions Condition 1: a pointed gastric tube, room-temperature distilled water, and injected at normal speed (approximately 3 ml/min); condition 2: a rounded gastric tube, 37°C distilled water, and slow injection (1.0 ml/min). (a) Photograph of the equipment used in the experiment (Periscan PIM-2); (b) diagram of the experimental procedure; s.c.: subcutaneous; (c) representative measurements of cremasteric arteriole BF in an anesthetized rat in condition 1; (d) representative measurement of cremasteric arteriole BF in an anesthetized rat in condition 2. au: arbitrary units.

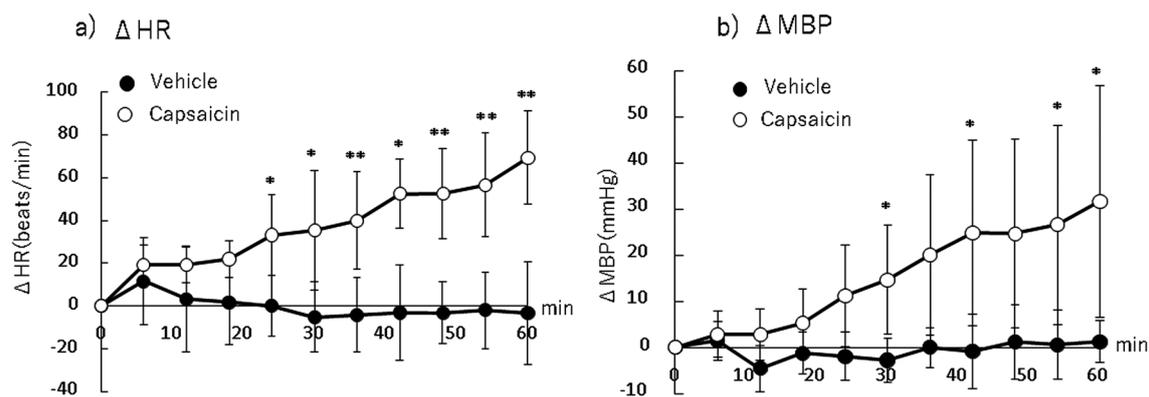


Fig. 3. Alterations in heart rate (HR) and mean blood pressure (MBP) in rats following a single gavage of 600 $\mu\text{g}/\text{kg}$ capsaicin with the method developed in this study. Changes in (a) HR and (b) MBP are shown for rats injected with capsaicin (cap; open symbols; $n=4$) or vehicle (filled symbols; $n=8$). Values are the means \pm SD. * $P<0.05$, ** $P<0.01$ indicate significant differences from vehicle.

rection. P -values ≤ 0.05 were considered significant.

Results

We measured the HR, MBP, and BF of the cremaster muscle arteriole under the two different conditions. The changes in HR and MBP in condition 1 (Fig. 1c) and in condition 2 (Fig. 1d) were dramatically different. During the baseline observation period, the HR was nearly constant (data not shown). In condition 1, the infusion of distilled water caused a transient, extreme increase in HR, which returned to the initial value at 30–40 min. This increase in HR observed in condition 1 was nearly eliminated in condition 2. Similarly, in condition 1, a temporary increase was observed in the MBP, and again, this elevation was nearly absent in condition 2 (Fig. 1d). Finally, in condition 1, there was a transient increase in cremaster arteriole BF soon after the injection of distilled water (Fig. 2c), and again, this change nearly disappeared in condition 2 (Fig. 2d).

In the next experiments, we used condition 2 for the gavage administration, because it caused no significant effects on HR, MBP, or BF. First, we measured the changes in HR and MBP after a single gavage administration of capsaicin, a pungent substance that activates sympathetic nerves (Fig. 3). The HR increased soon after a single gavage administration of 600 $\mu\text{g}/\text{kg}$ capsaicin. This effect was significantly different from the effect of the vehicle (3% ethanol, 10% tween80 in saline) at 24, 30, 36, 42, 48, 54, and 60 min after administration. Similarly, the administration of capsaicin increased the MBP. These changes were exhibited during the observation period (10 min), but they were significantly different from the effect of vehicle at 30, 42, 54, and 60 min after administration.

We also measured the BF in the cremaster arteriole

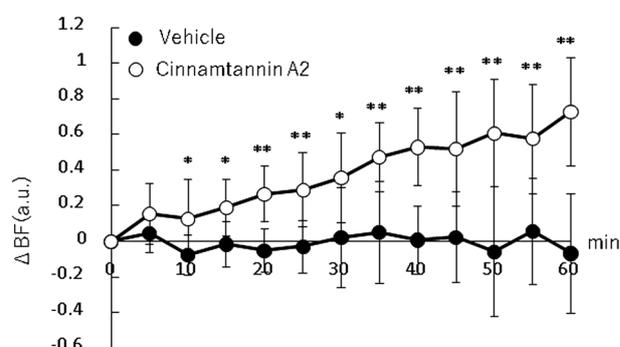


Fig. 4. Alterations in cremasteric arteriole blood flow (BF) in rats following a single gavage of 10 $\mu\text{g}/\text{kg}$ cinnamtannin A2 with the method developed in this study. Changes in BF are shown for rats injected with cinnamtannin A2 (A2, open symbols) or vehicle (filled symbols); $n=8$ per group. Values are the means \pm SD. * $P<0.05$, ** $P<0.01$ indicate significant differences from vehicle. au: arbitrary units.

after a single gavage administration of 10 $\mu\text{g}/\text{kg}$ cinnamtannin A2 (Fig. 4). Cremasteric BF increased soon after the gavage of cinnamtannin A2, and this change was maintained for 60 min. The BF was significantly different from the effect of vehicle (3% Tween80, 97% distilled water) at 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min after administration.

Discussion

Previous experimental and clinical studies have shown that HR can be changed by stimulating somatic afferent nerves, including the visceral nerve [5]. A cardiac reflex can be elicited with innocuous stimuli, such as mechanical [6] or thermal (i.e., cold or hot) stimulation [7]. Neurons in the rostral ventrolateral medulla (rVLM) receive convergent input from visceral organs. After

activating the rVLM, the excitatory neurotransmitter, glutamate, induces visceral sympathoexcitatory cardiovascular reflexes [8]. In the present study, marked transient increases were observed in the HR, MBP, and cremaster arteriole BF, after a gavage administration in condition 1 (Figs. 1c and 2c). These results suggested that a pointed gastric tube, distilled water at room temperature, and a rapid injection caused a cardiac response via the visceral afferents in the stomach.

Previous studies reported that food components with pungent or astringent attributes can alter hemodynamics. Capsaicin is a typical pungent substance known to bind to the transient receptor potential vanilloid 1 (TRPV1). Oral administration of capsaicin enhanced sympathetic nerve activity [9]. In association with sympathetic nervous activation, various studies have shown the effects of capsaicin on circulation. Chronic TRPV1 activation with dietary capsaicin was reported to improve endothelium-dependent vasorelaxation and reduce blood pressure [10]. In addition, procyanidins, which are (–)-epicatechin oligomers with a potent astringent taste, were shown to reduce the risk of cardiovascular diseases. Several intervention trials have demonstrated the hypotensive effects of dietary procyanidins [11, 12]. For example, we previously reported that repeated oral administration of a specific procyanidin fraction reduced the MBP in mice [13]. Moreover, we found that procyanidins enhanced sympathetic nerve activities [14].

It has been suggested that the oral administration of pungent and astringent substances could cause hemodynamic changes mediated by the stimulation of sensory nerves distributed in the gastrointestinal tract, including the oral cavity [15]. Because the cardiac reflex induced by gavage administration interferes with the hemodynamic responses to these chemicals, in the present study, we developed a new method for gavage administration that did not interfere with HR, MBP, or cremaster arteriole BF measurements in rats.

Our new method involved changing the gavage conditions by using a rounded, rather than a pointed gastric tube; warm, rather than room-temperature distilled water; and slow, rather than rapid injections. As a result, the striking changes in hemodynamics, due to stimulating the somatic afferent reflex, were nearly absent in the new conditions (Figs. 1d and 2d). We evaluated the validity of this method by measuring HR and MBP after a single gavage administration of pungent capsaicin (Fig. 3) and by measuring cremaster arteriole BF after a single gavage administration of astringent cinnamtannin A2 (Fig. 4). When we used the gavage method developed in this study, we showed that a single oral dose of capsaicin significantly increased the HR and MBP (Fig. 3), and a

gavage administration of cinnamtannin A2 significantly increased the cremaster arteriole BF (Fig. 4). These results confirmed the validity of the method.

In conclusion, we established a new gavage method that did not interfere with rat hemodynamics. Specifically, the HR, MBP, and cremaster arteriole BF were not significantly affected by this new method for gavage administration. We confirmed the validity of the developed method by demonstrating that we could measure the significant transient alterations in hemodynamics after administering a single gavage of a pungent or astringent substance.

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Conflict of Interest

The authors declare no conflict of interest associated with this study.

Author Contributions

KI, AS, RK, and TF conducted the experiments. YF and TF performed data analysis and interpretations. MS and NO wrote the initial draft of the manuscript. NO critically reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All authors agree to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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