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Short Communication

Variation of Capsaicin-Sensitive Motor Activities along the Rat Gastrointestinal Tract

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Abstract

Variation in motility may be a character to move gut contents. The aim of present study was to assess whether the rat upper gastrointestinal motilities were variable according to the segments or studied periods under systemic capsaicin treatment. Sedated rats were intubated with a catheter to feed a suspension containing both charcoal and radiochromium motility markers. Capsaicin in the doses of 0.5 mg kg^{-1}, 1 mg kg^{-1}, 5 mg kg^{-1} or vehicle were simultaneously injected via intraperitoneal route. They were sacrificed at 5 min or 30 min later and the whole gut was removed. Charcoal transit in the small intestine was computed while the radioactivities of stomach and ten equally divided small intestinal segments were counted to obtain the gastric emptying and geometric center of intestinal transit, respectively. Large dose treatment inhibited early gastric emptying ($p<0.05$), whereas late gastric emptying remained unchanged. Larger dose treatment inhibited charcoal represented transit in the early ($p<0.05$) and late periods ($p<0.01$). The intestinal transits seen with geometric center were almost similar to these of charcoal representation ($p<0.01$). In conclusion, capsaicin-sensitive gastric emptying changes with studied periods while intestinal transit is always inhibited at any period. We confirm the notion of variation in capsaicin-sensitive motor responses along the rat upper digestive organ.

Key Words: capsaicin, gastric emptying, gastrointestinal motility, gastrointestinal transit

Introduction

Hot chilli / pepper has long been a food additive/ preservative, appetizer and sometimes in herb medicine to treat a variety of disorders. For instance, ingestion of chilli pepper may effectively ameliorate constipation (2, 15). Capsaicin, the pungent ingredient in red peppers, is introduced as an analgesic to treat rheumatism, lumbago, neuralgia and flatulence (5). Capsaicin mainly simulates afferent nerves in releasing stored transmitters from nerve endings, hence this activity involves various levels of physiological and pathophysiological behaviors (2, 11, 21). Ingestion of capsaicin contained chilli delays human gastric emptying but its effect on orocecal transit remains unchanged (13). Forceful gavage of capsaicin in rats also retards late gastric emptying, whereas the early gastric emptying is not altered (16). Moreover, systemic administration of capsaicin effectively inhibits rat intestinal transit (23). It looks that the capsaicin-sensitive motor responses in gastrointestinal (GI) tract vary according to the studied organs, experimental periods and routes of administration. In the present study, we employed an orogastric feeding model in simultaneously measuring rat stomach and intestinal motilities to confirm the probable notion

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of organ variation in capsaicin-sensitive motor responses.

Materials and Methods

Animals

The experiment was conducted in accordance with the guide principles in the care and use of animals of National Yang-Ming University. It was also approved by this hospital. Adult Sprague-Dawley male rats aging 3-4 months and weighing 350-450 gm (Animal room, National Yang-Ming University) were housed under the controlled temperature and illumination. Prior to the motility study, animals were deprived of food but allowed free access to water *ad libitum* for 18 hours.

Measurement of Gastrointestinal Motility

Gastric emptying and intestinal transit were assessed by the propulsion of a non-nutrient test suspension within the GI tract (3). The main compositions of this suspension were $Na^{51}CrO_4$ (1 mCi=37 MBq, Dupont, NEN Research Products, USA) with a radioactivity 0.5 μCi ml^{-1} and 10% charcoal (Sigma, St. Louis, USA). It was diluted with 154 mM saline and continuously stirred with a shaker (Corning, USA) until instillation. Under conscious state studied rats were fed with this suspension via an orogastric catheter (ID: 1.67 mm, OD: 2.42 mm, PE-205, Clay-Adams, USA). The feeding amount for each animal was adjusted to 3 ml kg^{-1}. Additional air was added to flush the residual suspension remaining in the catheter into the stomach. When a successful feeding was achieved, the rats immediately received an intraperitoneal injection of either capsaicin or vehicle to study the motor response of GI tract. Five or thirty minutes after feeding rats were sacrificed with a guillotine. The stomach and small intestine were exposed by a laparotomy. Both cardiac and pyloric ends of stomach, and the distal end of small intestine were ligated. The stomach and small intestine were carefully removed outside. Charcoal transit is the percent of charcoal traveled length within small intestine divided by the total length of small intestine (4). Stomach and 10 equally divided small intestinal segments were separated and then both ends of every segment were sealed with a cauterical device (Bipolar coagulator 440E, Radionics, USA). Finally, all gastric and small intestinal segments were placed into the counting tubes to compute their radioactivities with a gammacounter (10/880 Plus, ICN Biomedicals, USA) for one minute. Gastric emptying is the percent of all intestinal radioactivity divided by the total radioactivity recovered from both stomach and ten intestinal segments. While the geometric center of intestinal transit is derived from the following formula (23).

Geometric center=Σ radioactivity of segment/ total intestinal radioactivity X segment number

Drugs and Preparations

Capsaicin (Sigma, St. Louis, USA) in this study was freshly diluted with a solvent vehicle containing 154 mM saline, absolute alcohol, and Tween 80 (Sigma, St. Louis, USA) in the volume ratio of 8:1:1 (7, 16). Capsaicin was then serially diluted into the concentrations of 0.5 mg ml^{-1}, 1 mg ml^{-1} and 5 mg ml^{-1}, respectively for the subsequent intraperitoneal administration. Regardless of receiving either capsaicin or vehicle, the amount of intraperitoneal injection for each animal was adjusted to 1 ml kg^{-1}. Each dose was repeated in six animals.

Statistical Analysis

All values were expressed as mean±SE, numerical data were analyzed by using a one-way analysis of variance (ANOVA) with post Dunnett's test. A p value less than 0.05 was considered to be significant.

Results

When the rats received a systemic vehicle treatment, the gastric emptying obtained at 5 min (early period) was 34.5±3.8% while the gastric emptyings of rats following capsaicin treatment in the doses of 0.5 mg ml^{-1}, 1 mg ml^{-1} and 5 mg ml^{-1} at the same period were 23.9±6.5%, 25.4±3.5% and 16.9±4.2%, respectively. The very large dose treatment inhibited gastric emptying ($p<0.05$). On the other hand, the gastric emptying of control rats studied at 30 min (late period) was 61.8±4.4% while the gastric emptyings of rats following various doses of capsaicin treatment at the same period were 51.8±10.1%, 81.1±3.6% and 81.2±7.7 %, respectively (NS). When the intestinal transit was represented with charcoal movement within lumen, the transits of rats received systemic vehicle treatment at early and late periods were 21.8±2.2% and 54.1±2.8%, respectively. Small doses of capsaicin treatment did not influence GI transit at any period, whereas large dose of capsaicin treatment inhibited transits in the early and late periods (Fig. 1). Insead, the geometric center represented intestinal transits for the vehicle treated rats were 3.52±0.37 and 4.91±0.18 during the early and late periods, respectively. The effects of capsaicin inhibited intestinal transit that was computed using geometric

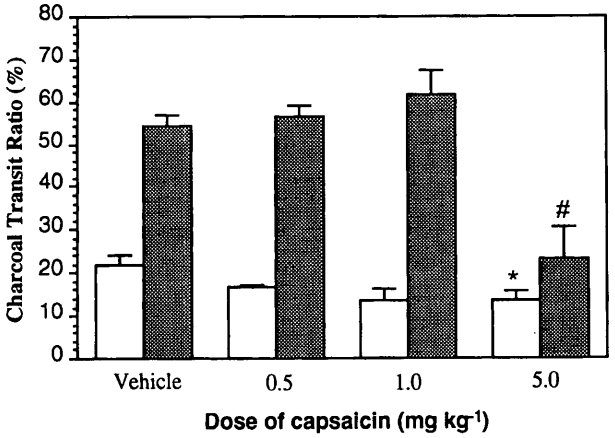

Fig. 1. Effect of systemical capsaicin treatment on charcoal represented intestinal transit that was obtained at 5 min (□) or 30 min (▩) after the feeding of charcoal marker. Capsaicin was simultaneously treated via i.p. injection, the rats received vehicle treatment served as controls. Each study was repeated in 6 animals, vertical bars are SE. Significant inhibitions of intestinal transit (*: vs. vehicle, $p<0.05$; #: vs. vehicle, $p<0.01$) were obtained in the rats receiving large dose of capsaicin treatment.

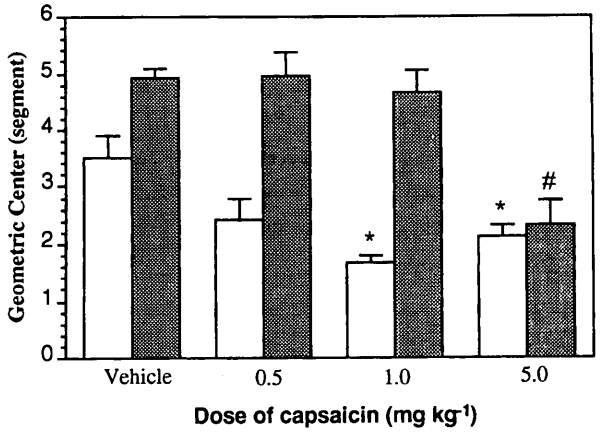

Fig. 2. Effect of systemical capsaicin treatment on geometric center represented intestinal transit that was obtained at 5 min (□) or 30 min (▩) after the feeding of radiochromium marker. Capsaicin was simultaneously treated via i.p. injection, the rats received vehicle treatment served as controls. Each study was repeated in 6 animals, vertical bars are SE. Significant inhibitions of intestinal transit (*, #: vs. vehicle, $p<0.01$) were obtained in the rats receiving moderate and large doses of capsaicin treatment.

center were almost the same as those of charcoal computation. For example, small doses of capsaicin did not influence GI transit at any period. Moderate and large doses of capsaicin treatment inhibited transits at early period ($p<0.01$) while the large dose of capsaicin treatment delayed transit in the late period ($p<0.01$, Fig. 2).

Discussion

The present study indicated that systemically administered capsaicin inhibited gastric emptying in the early period but the late gastric emptying remained unchanged. The sensory or afferent nervous system receives and transmits various informations and signals from both internal and external environments which in turn provide the organisms to adjust their homeostasis (11). Capsaicin is a very useful pharmacological tool to map the sensory innervation of tissues since it elicits nociception and depletes various neuropeptides from sensory neurons (11). The first identified neuropeptide via capsaicin stimulation has been substance P (SP) (14). Depleted SP does manifest a motor action on tissues. For example, capsaicin leads to a biphasic action on the stomach corpus muscle of rats, the low dose of capsaicin induced contraction can be blocked with SP antagonist while the high dose elicited inhibition is most likely a nonspecific relaxation ability of capsaicin (12, 17). These facts imply that released endogenous SP is involved in the motor stimulation. Usually, a small dose of capsaicin treatment can depletes stored SP, whereas a vary large dose (125 mg kg^{-1}) treatment results in 70-80% depletion (2). A very large dose of capsaicin in vitro treatment ultimately exhibits the specific membrane stability leading to motor suppression (1).

Nevertheless, the capsaicin elicited neuropeptide depletion is not confined to SP only. Other capsaicin-sensitive neuropeptides including neurokinin A, cholecystokinin, calcitonin gene-related peptide, vasoactive intestinal polypeptide, etc. have been released from the primary afferent neurons (11, 21). Even endogenous nitric oxide, a nonadrenergic noncholinergic inhibitory neurotransmitter candidate, is also activated via capsaicin treatment (1, 24). Thus both excitatory and inhibitory neuropeptides are already released together under capsaicin treatment. A functional antagonism does exist between the released contractile and relaxant neurotransmitters following capsaicin treatment while excitation always indicates the low affinity of relaxant peptides to their receptors (12). We are unknown whether all above neuropeptides have been released in turn to modulate GI motility following systemic capsaicin treatment. Using a balloon pressure monitor, intragastric instillation of capsaicin inhibits rat gastric motility (29). Similarly, Kang et al (16) found the delayed late gastric emptying. Capsaicin-sensitive afferents in duodenum also inhibit rat gastric emptying (25). The liquid gastric emptying is exactly dependent on the flow resistance through pylorus and the pressure gradient between duodenum and gastric corpus (18). Pylorus has been the richest SP binding site in gut particularly the pyloric circular muscles (26, 27). Perhaps the net result of one of capsaicin released

neuropeptides, SP, on gastric emptying depends on the final balance between SP induced gastric contraction against a spasmogenic pyloric activity exerted by SP itself. Since gastric emptying is a well coordinated procedure, present study shown the different gastric emptyings seen at two studied periods suggests that the ultimate coordination of capsaicin released neuropeptides on gastric emptying varies with time intervals. Some neuropeptides do manifest such characteristic of timing variation. For example, SP and neurokinin A enhance rat gastric emptying at 15-20 min but an inhibited emptying is seen at 3 min after feeding (8).

The pharmacological effect of capsaicin is partially mediated via sympathetic adrenergic reflex pathway. For example, peritoneal irritation activates the visceral capsaicin-sensitive afferent neurons which in turn lead to stimulation of efferent adrenergic neurons and finally the inhibitory effect on intestinal motility (9). Holzer et al (10) further confirmed that capsaicin-sensitive sensory neurons might be involved in sympathetic reflex inhibition of gastrointestinal propulsion. Extraluminal capsaicin treatment in an in vitro model also induces gastric relaxation which is mediated via capsaicin-sensitive sensory nerves and independent of released endogenous SP (30). This study further points out that capsaicin-sensitive sensory nerve is one of non-adrenergic non-cholinergic neural pathways. The subsequent gastric relaxation following capsaicin treatment is most likely to delay gastric emptying. Using two motility markers our study demonstrated the delayed intestinal transit, irrespective of studied periods. This result was in accordance with Miller et al (23). Capsaicin does inhibit small intestinal muscle strips (19, 20). An in vivo electromyographic recording also provides the capsaicin-sensitive inhibition (6). It remains unresolved whether the intestinal inhibition is mainly mediated by the dominant action of capsaicin-released inhibitory neuropeptides. At least the motor suppression of very large dose of capsaicin resulted membrane stability was impossible because only moderate dose (5 mg kg^{-1}) was employed in our study (1).

It can be expected that the capsaicin-sensitive endogenous peptides must have smilar chance to activate both stomach and intestinal receptors. However, the obtained motor responses were quite distinct in different organs and studied periods. We suggest that this variation represents the differences of the affinities of capsaicin-sensitive neuropeptides on their corresponding receptors, the receptor numbers, the timing variation of effective peptides and the anatomical characteristics along the GI tract. Motility variation has been present in the colon since proximal segment exhibits a different tone under peptide treatment in comparison with this of distal segment (28). This character also exists in different GI organs. Likewise, motilin is essential to produce migrating motor cycle on stomach than in small intestine (22). The character of motility variation appears beneficial to transport contents between regions of GI-tract (28). In conclusion, systemic capsaicin treatment inhibits early gastric emptying but the late emptying remains unchanged, intestinal transit is always inhibited at any period. These results confirm that the coordinated motor actions of capsaicin-released neuropeptides along the rat GI tract are variable.

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