Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 457 (2002) 169–176
www.elsevier.com/locate/ejphar

Effects of evodiamine on gastrointestinal motility in male rats

Chiu-Lung Wu[a], Chen-Road Hung[b], Full-Young Chang[c], Lie-Chwen Lin[d], K.-Y. Francis Pau[e], Paulus S. Wang[a,*]

[a] Department of Physiology, School of Medicine, National Yang-Ming University, Shih-Pai, Taipei 11221, Taiwan, ROC
[b] Department of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan 70101, Taiwan, ROC
[c] Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taipei 11221, Taiwan, ROC
[d] National Research Institute of Chinese Medicine, Taipei 11221, Taiwan, ROC
[e] Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, OR 97006, USA

Received 19 July 2002; received in revised form 25 October 2002; accepted 29 October 2002

Abstract

The effects of evodiamine on gastric emptying, gastrointestinal transit, and plasma levels of cholecystokinin (CCK) were studied in male rats. Evodiamine, isolated from the dry unripened fruit of Evodia rutaecarpa Bentham (a Chinese medicine named Wu-chu-yu), has been recommended for abdominal pain, acid regurgitation, nausea, diarrhea, and dysmenorrhea. Gastrointestinal motility was assessed in rats 15 min after intragastric instillation of a test meal containing charcoal and $Na_2^{51}CrO_4$. Gastric emptying was determined by measuring the amount of radiolabeled chromium contained in the small intestine as a percentage of the initial amount received. Gastrointestinal transit was evaluated by calculating the geometric center of distribution of the radiolabeled marker. Blood samples were collected for CCK radioimmunoassay (RIA). After administration of evodiamine (0.67–6.00 mg/kg), both gastric emptying and gastrointestinal transit were inhibited, whereas the plasma concentration of CCK was increased in a dose-dependent manner. The selective CCK_1 receptor antagonists, devazepide and lorglumide, effectively attenuated the evodiamine-induced inhibition of gastric emptying and gastrointestinal transit. L-365,260 (3*R*-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-*N*′ -(3-methylphenyl)-urea), a selective CCK_2 receptor antagonist, did not alter the evodiamine-induced inhibition of gastric emptying and gastrointestinal transit. These results suggest that evodiamine inhibits both gastric emptying and gastrointestinal transit in male rats via a mechanism involving CCK release and CCK_1 receptor activation.

Keywords: Evodiamine; Gastrointestinal motility; Cholecystokinin; Devazepide; Lorglumide; L-365,260

1. Introduction

Evodiamine, isolated from the dry unripened fruit of Evodia rutaecarpa Bentham (a Chinese medicine named Wu-chu-yu), has been recommended for abdominal pain, acid regurgitation, nausea, diarrhea, and dysmenorrhea (Chang and But, 1986) and is used as an analgesic, antiemetic, astringent, and antihypertensive drug in Chinese medicine (Tang and Eisenbrand, 1992; Yang et al., 1990). Evodiamine was reported to have a stomachic (a tonic for improving the appetite or digestion) action (Chang and But, 1986) and to inhibit intestinal transit (Yu et al., 1994), but the action of evodiamine on the gastrointestinal tract has not yet been extensively studied. In the present study we examined the effects of evodiamine on gastric emptying and intestinal transit in male rats.

The functions of cholecystokinin (CCK) include stimulation of pancreatic enzyme secretion and inhibition of gastric emptying (Debas et al., 1975; Jin et al., 1994), as well as suppression of food intake (Moran and McHugh, 1982). Two subtypes of CCK receptors, CCK_1 and CCK_2, have been reported (Moran et al., 1986). CCK_1 receptors are found mainly in peripheral tissues and CCK_2 receptors are expressed mainly in the central nervous system (Innis and Synder, 1980; Kopin et al., 1992; Pisegna et al., 1992; Wank et al., 1992; Silvente-Poirot et al., 1993). A number of studies have suggested that CCK_1 receptors play an important role in the inhibition of gastric emptying by amphetamine or endogenous and exogenous CCK (Doong

* Corresponding author. Tel.: +886-2-28267082; fax: +886-2-28264049.
E-mail address: pswang@ym.edu.tw (P.S. Wang).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02687-0

et al., 1998; Beglinger, 1994; Varga and Scarpignato, 1996).

The purpose of this study was to investigate (1) the effects of evodiamine on gastric emptying, gastrointestinal transit, and plasma CCK levels, and (2) the involvement of CCK receptors in the action of evodiamine on gastrointestinal motility in male rats by using antagonists of CCK_1, devazepide (Reidelberger and O'Rourke, 1989) or lorglumide (Makovec et al., 1986), and CCK_2, L-365,260 (3*R*-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-*N*′ -(3-methylphenyl)-urea) (Lu et al., 2000).

2. Methods

2.1. Animals

Male Sprague–Dawley rats weighing 250–300 g were housed in a temperature (22 ± 1 °C)- and light (6 a.m.–8 p.m.)-controlled environment and fed ad libitum with rat chow. Tap water was given ad libitum.

Animal protocols were approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. All animals received care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, Taiwan, ROC.

2.2. Experimental protocol

2.2.1. Effects of evodiamine and CCK on gastric emptying and gastrointestinal transit

2.2.1.1. Experiment 1. The rats were randomly divided into four groups ($n = 7$). They were fasted (with access to water) for 24 h before use. On the day of the experiment, they were injected intraperitoneally (i.p.) with 0, 0.67, 2.00, or 6.00 mg/kg of evodiamine dissolved in 1 ml of dimethyl sulfoxide (DMSO), 15 min before intragastric administration of a non-nutrient liquid meal. Fifteen minutes after the administration of the liquid meal, the rats were decapitated and gastrointestinal transit was measured. Blood samples were collected for CCK radioimmunoassay (RIA).

2.2.1.2. Experiment 2. The rats were randomly divided into four groups ($n = 7$). They were fasted (with access to water) for 24 h before use. On the day of the experiment, they were injected intraperitoneally (i.p.) with 0, 0.1, 1.0, or 10 μg/kg of sulfated cholecystokinin (26-33) (CCK-8) dissolved in 1 ml of saline, 15 min before intragastric administration of a non-nutrient liquid meal. Fifteen minutes after the administration of the liquid meal, the rats were decapitated and gastrointestinal transit was measured.

2.2.2. Effects of selective CCK receptor antagonists on evodiamine effects on gastric emptying and gastrointestinal transit

2.2.2.1. Experiment 1. Male rats were divided into four groups of seven animals each and fasted for 24 h before use. Before intragastric administration of a non-nutrient liquid meal, the animals were injected i.p. with the following compounds in a volume of 1 ml/kg: groups 1 and 2 received DMSO, groups 3 and 4 were injected with DMSO containing devazepide (a CCK_1 receptor antagonist) at doses of 0.3 and 3.0 mg/kg, respectively (15 min before intragastric administration), groups 2–4 also received 2.0 mg/kg of evodiamine in DMSO, group 1 was injected with DMSO for control (30 min before intragastric administration). Fifteen minutes after the administration of the liquid meal, the rats were decapitated and gastrointestinal transit was measured.

2.2.2.2. Experiment 2. Male rats were divided into four groups of seven animals each and fasted for 24 h before use. Before intragastric administration of a non-nutrient liquid meal, the animals were injected i.p. with the following compounds in a volume of 1 ml/kg: groups 1 and 2 received saline, groups 3 and 4 were injected with saline containing lorglumide (a CCK_1 receptor antagonist) at doses of 5 and 10 mg/kg, respectively (15 min before intragastric administration), groups 2–4 also received 2.0 mg/kg of evodiamine in DMSO, group 1 was injected with DMSO for control (30 min before intragastric administration). Fifteen minutes after the administration of the liquid meal, the rats were decapitated and gastrointestinal transit was measured.

2.2.2.3. Experiment 3. The procedure was identical to that in experiment 1, except that L-365,260 (a CCK_2 receptor antagonist) was used instead of devazepide.

2.2.2.4. Experiment 4. Male rats were divided into four groups of seven animals each and fasted for 24 h before use. Fifteen minutes before intragastric administration of a non-nutrient liquid meal, the animals were injected i.p. with the following compounds in a volume of 1 ml/kg: group 1 received saline, group 2 received 2.0 mg/kg of evodiamine in DMSO, group 3 received 3.0 mg/kg of devazepide in DMSO, and group 4 received 10 mg/kg of lorglumide in saline. Fifteen minutes after the administration of the liquid meal, the rats were decapitated and gastrointestinal transit was measured.

2.3. Measurement of gastric emptying and gastrointestinal transit

Gastric emptying and gastrointestinal transit were measured as described by Doong et al. (1998). Rats were intubated via a catheter (PE-205, ID 1.67 mm, OD 2.42 mm, Clay-Adam, Parsippany, NJ, USA) and physiological

saline (3 ml/kg) containing $Na_2^{51}CrO_4$ (0.5 μCi/ml) and 10% charcoal was administered intragastrically. The test meal was continuously stirred before intubation. Air (0.5 ml) was used to flush the residual charcoal suspension in the catheter into the rat. Fifteen minutes later, the rats were decapitated and the stomach and attached small intestine were immediately exposed by laparotomy. After ligation of the esophagogastric, gastroduodenal, and ileocaecal junctions, the stomach and small intestine were carefully removed and placed on a wooden board to observe the leading edge of the charcoal in the intestine. The small intestine was then divided into 10 equal segments and the radioactivity in the stomach and each segment of small intestine was measured in an automatic gamma counter (1470 Wizard, Pharmacia, Turku, Finland). Gastric emptying was measured by determining the amount of labeled chromium contained in the small intestine 15 min after intubation, expressed as a percentage of the amount given (Doong et al., 1998; Chen et al., 1997; Holzer, 1985). Gastrointestinal transit was assessed by analyzing the geometric center of distribution of the radioactivity through these 10 equal-length segments (Miller et al., 1981; Chang et al., 1994; Chen et al., 1995, 1997). The geometric center was calculated by summing the percentage of radioactivity measured in each segment multiplied by the segment number (the most proximal segment numbered 1).

2.4. Processing of plasma

After decapitation, blood samples were collected and mixed with ethylenediaminetetraacetic acid (EDTA) (1 mg/ml of blood) and aprotinin (500 kiu/ml of blood). Plasma was immediately prepared by centrifugation at $1000 \times g$ for 30 min at 4 °C and used for measurement of plasma CCK concentrations. The plasma samples were acidified with an equal volume of 1% trifluoroacetic acid, then centrifuged at $2600 \times g$ for 20 min at 4 °C. A SEP-PAK C_{18} cartridge (Waters Associates, Milford, MA, USA) was equilibrated with 60% acetonitrile in 1% trifluoroacetic acid (1 ml), followed by 1% trifluoroacetic acid (3 ml, three times), then the supernatant from the treated plasma sample was applied. After the cartridge was slowly washed with 1% trifluoroacetic acid (3 ml, twice), the peptide (bound material) was slowly eluted with 3 ml of 60% acetonitrile in 1% trifluoroacetic acid. The eluant was collected, evaporated in a Speed Vac concentrator (Salvant Instruments, Farmingdale, NY, USA), and the dried samples were stored at −80 °C and reconstituted with the appropriate assay buffer before RIA (Hwu et al., 1992; Doong et al., 1998).

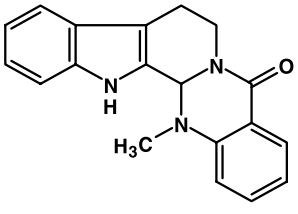
2.5. Chemical analysis

2.5.1. CCK RIA

The CCK concentration in extracted samples was measured by RIA using a rabbit anti-CCK antiserum supplied by Dr. K.Y. Francis Pau (Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, OR 97006, USA), and ^{3}H-CCK was purchased from Amersham International, Bucks, UK. In this RIA system, a known amount of unlabeled CCK in a total volume of 0.3 ml of 0.1% gelatin-PBS was incubated at 4 °C for 24 h with 100 μl of anti-CCK antiserum, diluted 1:2000 in normal rabbit serum, and 100 μl of [^{3}H]CCK (~ 8000 cpm). Triplicate standard curves with six points ranging from 1 to 1000 pg of unlabeled CCK were included in each assay. Two hundred microliters of anti-rabbit gamma-globulin was then added and the incubation was continued at 4 °C for 24 h. The assay tubes were then centrifuged at $1000 \times g$ for 20 min. The pellet was dissolved in 400 μl of 1 N NaOH, then 80 μl of 5 N HCl was added, the sample was mixed with 3 ml of liquid scintillation fluid, and the radioactivity was counted in an automatic counter (Wallac 1409, Pharmacia). The sensitivity of the CCK RIA was 8 pg of CCK per assay tube. The intra-assay and inter-assay coefficients of variation were 3% and 5%, respectively.

2.6. Drugs

Chemicals used in the study included ethylenediaminetetraacetic acid, aprotinin, sulfated cholecystokinin (26-33) (CCK-8) and trifluoroacetic acid, which were purchased from Sigma (St. Louis, MO, USA). Lorglumide sodium was purchased from Research Biochemicals International (Natick, MA, USA). Acetonitrile was purchased from Wako, Japan. $Na_2^{51}CrO_4$ was purchased from DuPont NEN Research Products, Boston, MA, USA. Devazepide and L-365,260 were kindly provided by ML Laboratories, Liverpool, UK. Evodiamine (Fig. 1) was prepared and provided by the National Research Institute of Chinese Medicine (Taipei, Taiwan, Republic of China). Evodiamine was first extracted from the dried fruit of Evodiae rutaecarpa with ethanol (60 °C for 16 h, four times), and then separated by column chromatography (Amberlite XAD-2). The purity, as determined by high-performance liquid chromatography with a UV detector (227 nm, Waters),



Evodiamine

($C_{19}H_{17}N_3O$, MW : 303.4)

Fig. 1. Structure and molecular weight of evodiamine.

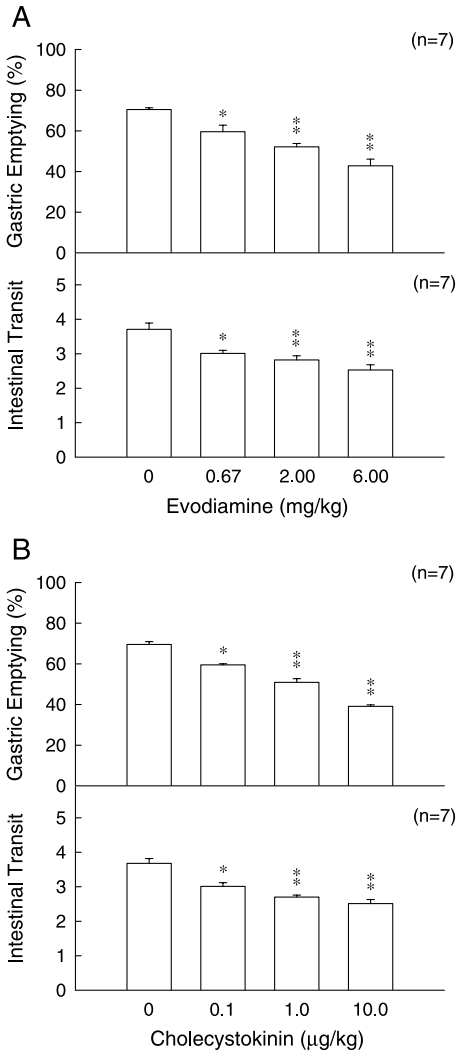


Fig. 2. (A) Effects of different doses of evodiamine on gastric emptying (upper panel) and gastrointestinal transit (lower panel) in male rats. Each column represents the mean ± S.E.M. *$P<0.05$ or **$P<0.01$, compared with control rats. (B) Effects of different doses of sulfated cholecystokinin (26-33) (CCK-8) on gastric emptying (upper panel) and gastrointestinal transit (lower panel) in male rats. Each column represents the mean ± S.E.M. *$P<0.05$ or **$P<0.01$, compared with control rats.

was greater than 99.8% (Chang and But, 1986; Chiou et al., 1992).

2.7. Statistical analysis

The data are expressed as the mean values ± S.E.M. The treatment means were tested for homogeneity using one-way analysis of variance, and the significance of any difference between means was tested using Duncan's multiple range test (Steel and Torrie, 1960). A difference between two means was considered to be statistically significant when P was less than 0.05.

3. Results

3.1. Effects of evodiamine and CCK on gastric emptying and gastrointestinal transit

3.1.1. Effects of evodiamine administration on gastric emptying and gastrointestinal transit

Administration of evodiamine (0.67, 2.00, and 6.00 mg/kg, i.p.) resulted in dose-dependent inhibition of gastric

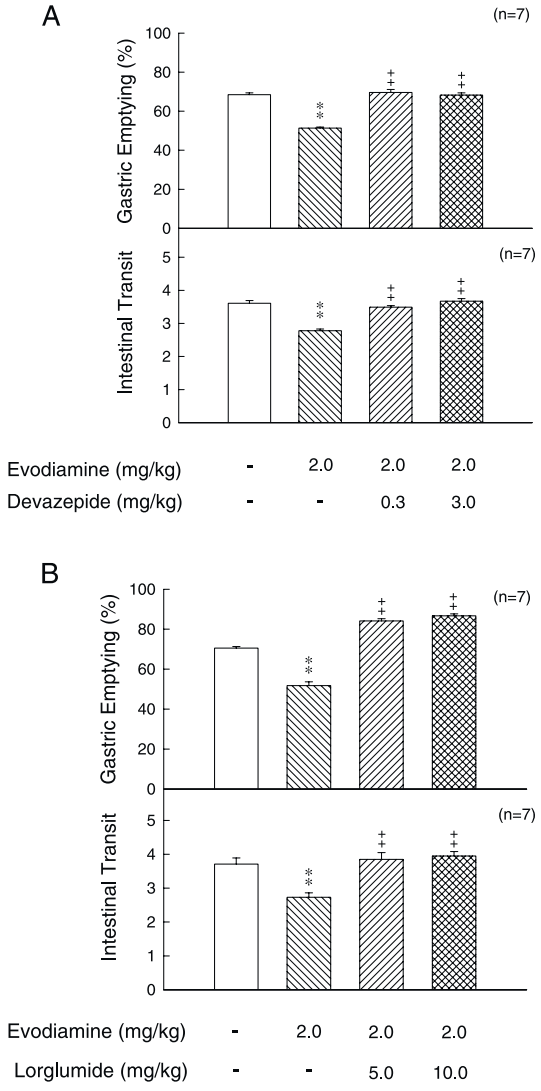


Fig. 3. (A) Effects of devazepide on the evodiamine-induced inhibition of gastric emptying (upper panel) and gastrointestinal transit (lower panel) in male rats. Each column represents the mean ± S.E.M. **$P<0.01$ compared with DMSO-injected rats. ++$P<0.01$ compared with the group treated with evodiamine, but not devazepide. (B) Effects of lorglumide on the evodiamine-induced inhibition of gastric emptying (upper panel) and gastrointestinal transit (lower panel) in male rats. Each column represents the mean ± S.E.M. **$P<0.01$ compared with DMSO-injected rats. ++$P<0.01$ compared with the group treated with evodiamine, but not lorglumide.

emptying ($P<0.05$ or <0.01) (Fig. 2A, upper panel) and also significantly inhibited gastrointestinal transit ($P<0.05$ or $P<0.01$) (Fig. 2A, lower panel).

3.1.2. Effects of sulfated CCK-8 administration on gastric emptying and gastrointestinal transit

Administration of sulfated CCK-8 (0.1, 1.0, and 10 μg/kg, i.p.) resulted in dose-dependent inhibition of gastric emptying ($P<0.05$ or $P<0.01$) (Fig. 2B, upper panel) and also significantly inhibited gastrointestinal transit ($P<0.05$ or $P<0.01$) (Fig. 2B, lower panel).

3.2. Effects of selective CCK antagonists on evodiamine effects on gastric emptying and gastrointestinal transit

3.2.1. Effects of devazepide on the evodiamine-mediated inhibition of gastric emptying and gastrointestinal transit

Treatment with devazepide (0.3 or 3 mg/kg) significantly reduced ($P<0.01$) the evodiamine-induced inhibition of gastric emptying (Fig. 3A, upper panel). Treatment with devazepide (0.3 or 3 mg/kg) also significantly blocked ($P<0.01$) the evodiamine-induced inhibition of gastrointestinal transit (Fig. 3A, lower panel).

3.2.2. Effects of lorglumide on the evodiamine-mediated inhibition of gastric emptying and gastrointestinal transit

Treatment with lorglumide (5 or 10 mg/kg) significantly reduced ($P<0.01$) the evodiamine-induced inhibition of gastric emptying (Fig. 3B, upper panel). Treatment with lorglumide (5 or 10 mg/kg) also significantly blocked ($P<0.01$) the evodiamine-induced inhibition of gastrointestinal transit (Fig. 3B, lower panel).

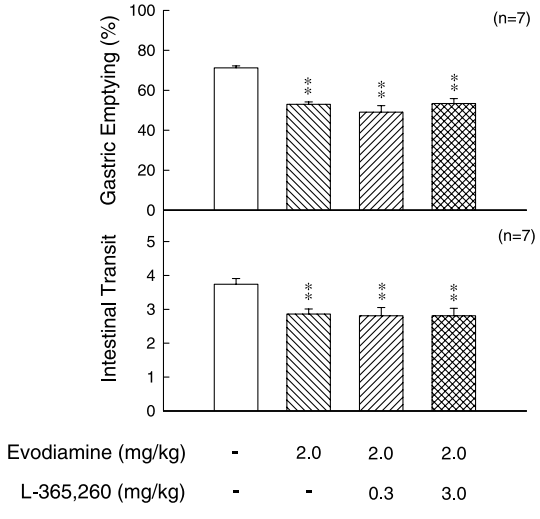


Fig. 4. Effects of L-365,260 on the evodiamine-induced inhibition of gastric emptying (upper panel) and gastrointestinal transit (lower panel) in male rats. Each column represents the mean ± S.E.M. $^{**}P<0.01$, compared with vehicle-injected rats.

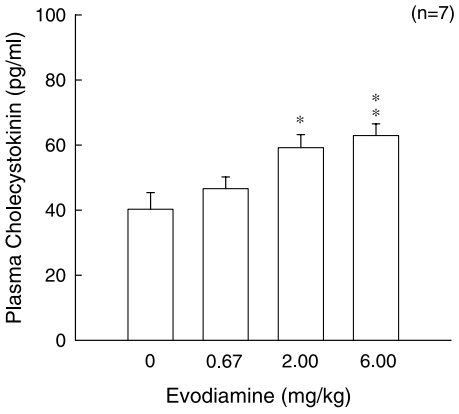


Fig. 5. Effects of evodiamine administration on the plasma cholecystokinin concentration in male rats. Radioimmunoassay was used to measure the plasma cholecystokinin concentrations in the rats. Each column represents the mean ± S.E.M. $^{*}P<0.05$ or $^{**}P<0.01$, compared with the control rats.

3.2.3. Effects of L-365,260 on the evodiamine-mediated inhibition of gastric emptying and gastrointestinal transit

L-365,260 (0.3 or 3 mg/kg) had no effect on evodiamine-induced gastric emptying or gastrointestinal transit (Fig. 4).

3.2.4. Effects of devazepide and lorglumide on gastric emptying and gastrointestinal transit

Administration of high-dose devazepide (3 mg/kg) or lorglumide (10 mg/kg) did not alter the gastric emptying or gastrointestinal transit.

3.3. Effects of evodiamine administration on the plasma CCK concentration

Administration of evodiamine (0.67, 2.00, and 6.00 mg/kg i.p.) resulted in a dose-dependent increase in the plasma CCK concentration (46.6 ± 3.6, 59.2 ± 4.0, and 62.9 ± 3.6 pg/ml, respectively, versus 40.3 ± 5.1 pg/ml for the control group, $n=7$) (Fig. 5).

4. Discussion

These results show that (1) evodiamine treatment inhibited gastric emptying and gastrointestinal transit in male rats, (2) intraperitoneally administered evodiamine increased the plasma CCK concentration in a dose-dependent manner, (3) selective CCK_1 receptor antagonists, devazepide and lorglumide, effectively attenuated evodiamine-induced inhibition of gastric emptying and gastrointestinal transit, and (4) a selective CCK_2 receptor antagonist, L-365,260, had no effect on evodiamine-induced inhibition of gastric emptying or gastrointestinal transit.

Wu-chu-yu is a Chinese traditional medicine used for migraine and nausea with vomiting as characteristic symptom. Evodiamine is one of the major components of Wu-

chu-yu. Previous studies have shown that evodiamine affects blood pressure, anoxia, and body temperature (Chiou et al., 1992; Yamahara et al., 1989; Tsai et al., 1995). Evodiamine has been recommended for abdominal pain, acid regurgitation, nausea, diarrhea and dysmenorrhea (Chang and But, 1986) and is used as an analgesic, antiemetic, astringent, and antihypertensive drug in Chinese medicine (Tang and Eisenbrand, 1992; Yang et al., 1990). The possible anti-inflammatory effects of evodiamine were examined by assessing the effects on nitric oxide production in the murine macrophage (Chiou et al., 1997). Results indicated that evodiamine indeed influences many organs and tissues, and may become a useful medicine in the future. The present study demonstrated that after administration of evodiamine, both gastric emptying and gastrointestinal transit were inhibited.

Several studies have shown that evodiamine has a stomachic action (Chang and But, 1986) and inhibits intestinal transit (Yu et al., 1994). This inhibitory effect of evodiamine was dose dependent. The effect of evodiamine was apparent within 15 min, reached a maximum at 30–60 min, and was reversed at 180 min (Yu et al., 1994). In our experiments, after intraperitoneal injection of evodiamine in male rats, both gastric emptying and gastrointestinal transit were inhibited, and the inhibitory effect of evodiamine was dose dependent.

It is well known that the gastrointestinal tract is a complicated system, in which many factors including nerves, neurotransmitters, neuropeptides, and hormones modulate its activities or functions (Burks, 1981). In studying the possible mechanism of evodiamine action on gastric emptying and gastrointestinal transit, previous studies suggested that α- and β-adrenoceptors were not involved in the inhibitory effect of evodiamine on intestinal transit (Yu et al., 1994). CCK secretion in rats is inhibited by pancreatic proteases and bile acids in the intestine. It has been hypothesized that the inhibition of CCK release caused by pancreatic proteases is due to proteolytic inactivation of a CCK-releasing peptide (CCK-RP) present in intestinal secretions (Liddle, 1995; Spannagel et al., 1996). CCK-RP is secreted into the proximal small intestine and is inactivated by trpsin. Postprandially, when food enters the duodenum, protein binds to trypsin and prevents CCK-RP from being inactivated. CCK-RP stimulates CCK cells in the duodenum to release CCK into the bloodstream (Herzig et al., 1996; Herzig, 1998; Li et al., 2000). A possibility is that the inhibition of gastric emptying and gastrointestinal transit induced by evodiamine is due to evodiamine acting in the intestinal lumen as a CCK-RP, and then CCK-RP stimulates CCK cells in the duodenum to release CCK into blood stream. Our data confirmed that the plasma CCK concentration was increased in a dose-dependent manner by evodiamine administration and that exogenous CCK-8 showed the same effects on the inhibition of gastric emptying and gastrointestinal transit in a dose-dependent manner. Therefore, the results suggest that the inhibition of gastric emptying and gastrointestinal transit is due to the stimulation of CCK release.

CCK slows gastric emptying in both laboratory animals and humans (Chey et al., 1970; Debas et al., 1975; Anika, 1982; Mangel and Koegel, 1984; Jin et al., 1994). Inhibition of gastric emptying is mediated by CCK-induced activation of an inhibitory vago-vagal reflex involving vasoactive intestinal peptide-induced relaxation of the gastric fundus (Grider, 1994). CCK inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats (Moran et al., 1987; Raybould and Taché, 1988; Moriarty et al., 1997). Postprandial CCK has been suggested as an important mediator of disruption of migrating myoelectric complexes after a meal (Ruckebusch and Fioramonti, 1975). Exogenous polyamines disrupt intestinal migrating myoelectric complexes through the release of CCK acting at CCK_1 and CCK_2 receptors, and endogenous polyamines are involved in the postprandial control of intestinal motility (Fioramonti et al., 1994). CCK-mediated motor changes after a meal are due to stimulation of peripheral CCK_2 receptors. CCK also induces the release of central CCK, which, acting through CCK_1 receptors, participates in the disruption of migrating myoelectric complexes (Rodriguez-Membrilla et al., 1995). In the present study, evodiamine treatment of male rats resulted in a parallel increase in the plasma CCK concentration and a marked decrease in gastric emptying and gastrointestinal transit, suggesting that the inhibition of gastric emptying and gastrointestinal transit induced by evodiamine might be related to the stimulation of CCK release. We therefore initiated the CCK antagonist trials.

CCK delays the gastric emptying of liquids by stimulation of CCK_1 receptors (Beglinger, 1994; Moran et al., 1994; Varga and Scarpignato, 1996). It is also suggested that CCK inhibits gastric emptying in rats by causing contraction of the pyloric sphincter, which is prevented by CCK_1 receptor antagonists (Murphy et al., 1987; Margolis et al., 1989). However, CCK_1 and CCK_2 receptor mRNAs have been detected in the rat stomach (Monstein et al., 1996) and the role of CCK_2 in mediating gastric motility has not been established. In the present study, devazepide, lorglumide and L-365,260 were used to differentiate the CCK receptor subtypes mediating the evodiamine-induced inhibition of gastric emptying and gastrointestinal transit. Our data show that devazepide and lorglumide blocked the evodiamine-induced inhibition of gastric emptying and inhibition of gastrointestinal transit, whereas devazepide or lorgiumide alone had no effect on either function. In contrast, L-365,260 had no effect on the inhibition of gastric emptying or gastrointestinal transit caused by evodiamine.

In summary, the present investigation suggests that evodiamine administration inhibits gastric emptying and gastrointestinal transit, which occurred concomitantly with an increase in plasma CCK concentration. The results also suggest that CCK_1, but not CCK_2, receptors are involved in the evodiamine-induced inhibition of gastric emptying. These observations are consistent with the concept that

evodiamine, in association with CCK, plays an important role in regulating gastric motility and gastrointestinal transit.

Acknowledgements

The present study was supported by a research grant from the National Science Council, Taiwan, ROC (No. NSC89-2315-B075-011).

Devazepide and L-365,260 were kindly provided by ML Laboratories, Liverpool, UK.

References

Anika, M.S., 1982. Effects of cholecystokinin and caerulein on gastric emptying. Eur. J. Pharmacol. 85, 195–199.

Beglinger, C., 1994. Effect of cholecystokinin on gastric motility in humans. Ann. N.Y. Acad. Sci. 713, 219–225.

Burks, T., 1981. Actions of drugs on gastrointestinal motility. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract, vol. 1. Raven Press, New York, pp. 495–516.

Chang, H.M., But, P.P.H., 1986. Wuzhuyu. In: Chang, H.M., But, P.P.H. (Eds.), Pharmacology and Applications of Chinese Materia Medica. World Scientific Publishing, Singapore, pp. 605–609.

Chang, F.Y., Lee, S.D., Yeh, G.H., Wang, P.S., 1994. Comparison of two orogastric feeding markers for measuring gastrointestinal motor functions in rats. Pharmacology 49, 151–158.

Chen, T.S., Doong, M.L., Chang, F.Y., Lee, S.D., Wang, P.S., 1995. Effects of sex steroid hormones on gastric emptying and gastrointestinal transit in rats. Am. J. Physiol. 268, G171–G176.

Chen, T.S., Doong, M.L., Wang, S.W., Tsai, S.C., Lu, C.C., Shih, H.C., Chen, Y.H., Chang, F.Y., Lee, S.D., Wang, P.S., 1997. Gastric emptying and gastrointestinal transit during lactation in rats. Am. J. Physiol. 272, G626–G631.

Chey, W.Y., Hitanant, S., Hendricks, J., Lorber, S.H., 1970. Effect of secretin and cholecystokinin on gastric emptying and gastric output in man. Gastroenterology 58, 820–827.

Chiou, W.F., Chou, C.J., Shum, A.Y., Chen, C.F., 1992. The vasorelaxant effect of evodiamine in rat isolated mesenteric arteries: mode of action. Eur. J. Pharmacol. 215, 277–283.

Chiou, W.F., Sung, Y.J., Liao, J.F., Shum, A.Y., Chen, C.F., 1997. Inhibitory effect of dehydroevodiamine and evodiamine on nitric oxide production in cultured murine macrophages. J. Nat. Prod. 60, 708–711.

Debas, H.T., Farooq, O., Grossman, M.I., 1975. Inhibition of gastric emptying is a physiological action of cholecystokinin. Gastroenterology 68, 1211–1217.

Doong, M.L., Lu, C.C., Kau, M.M., Tsai, S.C., Chiao, Y.C., Chen, J.J., Yeh, J.Y., Lin, H., Huang, S.W., Chen, T.S., Chang, F.Y., Wang, P.S., 1998. Inhibition of gastric emptying and intestinal transit by amphetamine through a mechanism involving an increased secretion of CCK in male rats. Br. J. Pharmacol. 124, 1123–1130.

Fioramonti, J., Fargeas, M.J., Bertrand, V., Pradayrol, L., Buéno, L., 1994. Induction of postprandial intestinal motility and release of cholecystokinin by polyamines in rats. Am. J. Physiol. 267, G960–G965.

Grider, J.R., 1994. Role of cholecystokinin in the regulation of gastrointestinal motility. J. Nutr. 124, 1334S–1339S.

Herzig, K.H., 1998. Cholecystokinin- and secretin-releasing peptides in the intestine—a new regulatory interendocrine mechanism in the gastrointestinal tract. Regul. Pept. 73, 89–94.

Herzig, K.H., Schön, I., Tatemoto, K., Ohe, Y., Li, Y., Fölsch, U.R., Owyang, C., 1996. Diazepam binding inhibitor is a potent cholecystokinin-releasing peptide in the intestine. Proc. Natl. Acad. Sci. 93, 7927–7932.

Holzer, P., 1985. Stimulation and inhibition of gastrointestinal propulsion induced by substance P and substance K in the rat. Br. J. Pharmacol. 86, 305–312.

Hwu, C.M., Tsai, S.C., Lau, C.P., Pu, H.F., Wang, T.L., Chiang, S.T., Wang, P.S., 1992. Increased concentrations of atrial and plasma atrial natriuretic peptide in castrated male rats. Life Sci. 52, 205–212.

Innis, R.B., Synder, S.H., 1980. Distinct cholecystokinin receptors in brain and pancreas. Proc. Natl. Acad. Sci. U. S. A. 77, 6917–6921.

Jin, H.O., Lee, K.Y., Chang, T.M., Chey, W.Y., Dubois, A., 1994. Physiological role of cholecystokinin on gastric emptying and acid output in dogs. Dig. Dis. Sci. 39, 2306–2314.

Kopin, A.S., Lee, Y.M., McBride, E.W., Miller, L.J., Lu, M., Lin, H.Y., Kolakowski, L.F., Beinborn, M., 1992. Expression cloning and characterization of the canine parietal cell gastrin receptor. Proc. Natl. Acad. Sci. U. S. A. 89, 3605–3609.

Li, Y., Hao, Y., Owyang, C., 2000. Diazepam-binding inhibitor mediates feedback regulation of pancreatic secretion and postprandial release of cholecystokinin. J. Clin. Invest. 105, 351–359.

Liddle, R.A., 1995. Regulation of cholecystokinin secretion by intraluminal releasing factors. Am. J. Physiol. 269, G319–G327.

Lu, L., Huang, M., Liu, Z., Ma, L., 2000. Cholecystokinin-B receptor antagonists attenuate morphine dependence and withdrawal in rats. NeuroReport 11, 829–832.

Makovec, F., Bani, M., Chiste, R., Revel, L., Rovati, L.C., Setnikar, L., 1986. Different peripheral and central antagonistic activity of new glutaramic acid derivatives on satiety induced by cholecystokinin in rats. Regul. Pept. 16, 281–290.

Mangel, A.W., Koegel, A., 1984. Effects of peptides on gastric emptying. Am. J. Physiol. 246, G342–G345.

Margolis, R.L., Moran, T.H., McHugh, P.R., 1989. In vitro response of rat gastrointestinal segments to cholecystokinin and bombesin. Peptides 10, 157–161.

Miller, M.S., Galligan, J.J., Burks, T.F., 1981. Accurate measurement of intestinal transit in the rat. J. Pharmacol. Methods 6, 211–217.

Monstein, H.J., Nylander, A.G., Salehi, A., Chen, D., Lundquist, I., Hakanson, R., 1996. Cholecystokinin-A and cholecystokinin-B/gastrin receptor mRNA expression in the gastrointestinal tract and pancreas of rat and man. A polymerase chain reaction study. Scand. J. Gastroenterol. 31, 383–390.

Moran, T.H., McHugh, P.R., 1982. Cholecystokinin suppresses food intake by inhibiting gastric emptying. Am. J. Physiol. 242, R491–R497.

Moran, T.H., Robinson, P.H., Goldrich, M.S., McHugh, P.R., 1986. Two brain cholecystokinin receptors: implications for behavioral actions. Brain Res. 362, 175–179.

Moran, T.H., Smith, G.P., Hostetler, A.M., McHugh, P.R., 1987. Transport of cholecystokinin (CCK) binding sites in subdiaphragmatic vagal branches. Brain Res. 415, 149–152.

Moran, T.H., Kornbluh, R., Moore, K., Schwartz, G.J., 1994. Cholecystokinin inhibits gastric emptying and contracts the pyloric sphincter in rats by interacting with low affinity CCK receptor sites. Regul. Pept. 52, 165–172.

Moriarty, P., Dimaline, R., Thompson, D.G., Dockray, G.J., 1997. Characterization of cholecystokinin A and cholecystokinin B receptors expressed by vagal afferent neurons. Neuroscience 79, 905–913.

Murphy, R.B., Smith, G.P., Gibbs, J., 1987. Pharmacological examination of cholecystokinin (CCK-8)-induced contractile activity in the rat isolated pylorus. Peptides 8, 127–134.

Pisegna, J.R., De Weerth, A., Huppi, K., Wank, S.A., 1992. Molecular cloning of human brain and gastric cholecystokinin receptor: structure, functional expression and chromosomal localization. Biochem. Biophys. Res. Commun. 189, 296–303.

Raybould, H.E., Taché, Y., 1988. Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. Am. J. Physiol. 255, G242–G246.

Reidelberger, R.D., O'Rourke, M.F., 1989. Potent cholecystokinin antagonist L-364,718 stimulates food intake in rats. Am. J. Physiol. 257, R1512–R1518.

Rodriguez-Membrilla, A., Martinez, V., Vergara, P., 1995. Peripheral and central cholecystokinin receptors regulate postprandial intestinal motility in the rat. J. Pharmacol. Exp. Ther. 275, 486–493.

Ruckebusch, M., Fioramonti, J., 1975. Electrical spiking activity and propulsion in small intestine in fed and fasted rats. Gastroenterology 68, 1500–1508.

Silvente-Poirot, S., Dufresne, M., Vaysse, N., Fourmy, D., 1993. The peripheral cholecystokinin receptors. Eur. J. Biochem. 215, 513–529.

Spannagel, A.W., Green, G.M., Guan, D., Liddle, R.A., Faull, K., Reeve, J.R., 1996. Purification and characterization of a luminal cholecystokinin-releasing factor from rat intestinal secretion. Proc. Natl. Acad. Sci. 93, 4415–4420.

Steel, R.D., Torrie, J.H., 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.

Tang, W., Eisenbrand, G., 1992. Evodia rutaecarpa (Juss.) Benth. In: Tang, W., Eisenbrand, G. (Eds.), Chinese Drugs of Plant Origin. Springer Verlag, New York, pp. 509–514.

Tsai, T.H., Lee, T.F., Chen, C.F., Wang, L.C., 1995. Thermoregulatory effects of alkaloids isolated from Wu-chu-yu in afebrile and febrile rats. Pharmacol. Biochem. Behav. 50, 293–298.

Varga, G., Scarpignato, C., 1996. Camostate- and caerulein-induced delay of gastric emptying in the rat: effect of CCK receptor antagonists. Eur. J. Pharmacol. 306, 153–159.

Wank, S.A., Pisegna, J.R., De Weerth, A., 1992. Brain and gastrointestinal cholecystokinin receptor family: structure and functional expression. Proc. Natl. Acad. Sci. U. S. A. 89, 8691–8695.

Yamahara, J., Yamada, T., Kitani, T., Naitoh, Y., Fujimura, H., 1989. Anti-anoxic action of evodiamine, an alkaloid in Evodia rutaecarpa fruit. J. Ethnopharmacol. 27, 185–192.

Yang, M.C.M., Wu, S.L., Kuo, J.S., Chen, C.F., 1990. The hypotensive and negative chronotropic effects of dehydroevodiamine. Eur. J. Pharmacol. 182, 537–542.

Yu, L.L., Liao, J.F., Chen, C.F., 1994. Effect of the crude extract of Evodiae fructus on the intestinal transit in mice. Planta Med. 60, 308–312.