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# Inhibition of gastric emptying and intestinal transit by amphetamine through a mechanism involving an increased secretion of CCK in male rats

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**1** The effect of amphetamine on gastrointestinal (GI) transit and the plasma levels of cholecystokinin (CCK) were studied in male rats.

**2** Gastric emptying was inhibited both acutely and chronically by the administration of amphetamine. GI transit was decreased by the acute administration of amphetamine but not affected by the chronic administration of amphetamine.

**3** Plasma CCK levels were increased dose-dependently by amphetamine.

**4** Proglumide, a CCK receptor antagonist, prevented amphetamine-induced inhibition of gastric emptying and the decrease in GI transit in male rats.

**5** The selective $CCK_A$ receptor antagonist, lorglumide, dose-dependently attenuated the amphetamine-induced inhibition of gastric emptying in male rats. In contrast, the selective $CCK_B$ receptor antagonist, PD 135,158, did not reverse the effect of amphetamine on gastric emptying.

**6** Both lorglumide and PD 135,158 reversed the inhibitory effect of amphetamine on GI transit in male rats.

**7** These results suggest that amphetamine-induced inhibition of gastric emptying and intestinal transit is due in part to a mechanism associated with the hypersecretion of endogenous CCK.

**Keywords:** Amphetamine; cholecystokinin (CCK); gastric emptying; gastrointestinal (GI) transit; geometric centre; lorglumide; proglumide; PD 135,158

## Introduction

Amphetamine is an effective anorectic agent and, historically, amphetamine has been used in the clinical treatment of obesity with emphasis on appetite control (Colton *et al*., 1943; Tainter, 1944; Harris *et al*., 1947). The drug acts to reduce appetite and suppress food intake via an indirect action on dopaminereceptors and/or $\beta$-adrenoceptors within the lateral hypothalamus (Booth, 1968; Leibowitz, 1975; Cole, 1978). Most studies have focused largely on the feeding behaviour and the central mechanism of action of amphetamine. However, signals generated from the gastrointestinal (GI) tract may play an important role in regulation of food intake. A number of studies have previously shown that the anorexic action of amphetamine may reflect, in part, an inhibitory action on gastric emptying (Vanliere & Sleeth, 1939; Bridges *et al*., 1975; Hull *et al*., 1993). Few studies have investigated the potential effects of amphetamine on alteration of small intestinal transit and circulating gut hormones levels.

The gut hormone cholecystokinin (CCK; Johnson, 1991) is well known to inhibit gastric emptying (Debas *et al*., 1975; Jin *et al*., 1994). Moreover, CCK suppresses food intake by inhibiting gastric emptying (Moran & McHugh, 1982). Therefore, it is of interest to clarify whether plasma CCK levels are altered by amphetamine in the peripheral circulation system and, if so, if the changes are related to amphetamine-induced inhibition of GI function.

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Two types of CCK receptors, $CCK_A$ and $CCK_B$, have been classified (Moran *et al*., 1986). $CCK_A$ receptors are most abundant in peripheral tissues. $CCK_B$ receptors are found predominantly in the central nervous system (Innis & Synder, 1980; Kopin *et al*., 1992; Pisegna *et al*., 1992; Wank *et al*., 1992; Silvente-Poirot *et al*., 1993). Endogenous and exogenous CCK delays gastric emptying of liquids through stimulation of $CCK_A$ receptors (Beglinger, 1994; Varga & Scarpignato, 1996). Proglumide, a CCK receptor antagonist, binds preferentially to $CCK_A$ receptors (Hahne *et al*., 1981), and has been shown to inhibit the effects of CCK on the gallbladder, stomach, ileum and central nervous system (Gardner & Jensen, 1984). Lorglumide, a selective $CCK_A$ receptor antagonist, is more potent than proglumide (Makovec *et al*., 1986). PD 135,158 is a selective $CCK_B$ receptor antagonist (Hughes *et al*., 1990). The purpose of the present study was to investigate the effects of amphetamine on GI transit. The CCK receptor antagonists, proglumide, lorglumide and PD 135,158 were employed to clarify the role of CCK in regulating the amphetamine effects on GI transit and to determine which CCK receptor type is involved.

## Methods

### *Animals*

Male Sprague-Dawley rats weighing 300–350 g were housed in a temperature controlled ($22 \pm 1^{\circ}C$) environment with 14 h

of artificial illumination daily (06 h 00 min – 20 h 00 min). All rats were given rat chow and tap water *ad libitum*.

### *Experiment 1*

Rats were divided into three groups and fasted for 24 h before use. Rats in the first group were injected intraperitoneally (i.p.) with saline once daily for ten days. Rats in the second group were injected i.p. with 3 mg $kg^{-1}$ amphetamine 30 min before gastric intubation of a non-nutrient liquid meal on the experimental day. Rats in the third group were injected i.p. with 1 mg $kg^{-1}$ amphetamine once daily for ten days before use. Fifteen minutes after the administration of the liquid meal, each rat was decapitated and GI transit measured.

### *Experiment 2*

Rats were fasted for 24 h before use. Each was injected i.p. with saline or amphetamine (0.75, 1.5 or 3 mg $kg^{-1}$) 30 min before gastric intubation of a non-nutrient liquid meal. Fifteen minutes after the administration of the liquid meal, each rat was decapitated and blood samples collected.

### *Experiment 3*

Rats were divided into four groups and fasted for 24 h before use. They received an i.p. injection of normal saline or proglumide sodium (10 mg $kg^{-1}$) 10 min before an i.p. injection of normal saline or amphetamine (3 mg $kg^{-1}$). Thirty minutes later, rats received the test meal. Fifteen minutes after the administration of the liquid meal, rats were decapitated and GI transit measured.

### *Experiment 4*

Rats were fasted for 24 h before use. They received an i.p. injection of normal saline, 3, 10 or 30 mg $kg^{-1}$ proglumide sodium 10 min before an i.p. injection of amphetamine (3 mg $kg^{-1}$). Thirty minutes later, rats received the test meal. Fifteen minutes after administration of the liquid meal, rats were decapitated and GI transit measured.

### *Experiment 5*

Rats were divided into four groups and fasted for 24 h before use. They received an i.p. injection of normal saline or one of the selective CCK receptor antagonists, lorglumide sodium (a $CCK_A$ receptor antagonist, 5 or 10 mg $kg^{-1}$) and PD 135,158 (N-methyl-D-glucamine; a $CCK_B$ receptor antagonist, 5 or 10 mg $kg^{-1}$) 10 min before an i.p. injection of normal saline or amphetamine (3 mg $kg^{-1}$). Thirty minutes later, rats received the test meal. Fifteen minutes after administration of the liquid meal, rats were decapitated and GI transit measured.

### *Measurement of gastric emptying and gastrointestinal transit*

On the day of the experiment (around 9 am), conscious rats were intubated via a catheter (PE-205, i.d.: 1.67 mm, o.d.: 2.42 mm, Clay-Adam, Parsippany, NJ, U.S.A.) with physiological saline (3 ml $kg^{-1}$) containing $Na_2{}^{51}CrO_4$ (0.5 $\mu$Ci $ml^{-1}$) and 10% charcoal. The test meal was continuously stirred before intubation. Additional air (0.5 ml) was added to flush the residual charcoal suspension remaining in the catheter into the rat. Fifteen minutes later the rats were decapitated. The stomach and attached small intestine were immediately exposed by a laparotomy. After ligation of the oesophagogastric, gastroduodenal and ileocaecal junctions, the whole stomach and small intestine were carefully mobilized and placed on a wooden board to observe the front which indicated the leading edge of charcoal moving within the intestine. The small intestine was then divided equally into 10 segments. The radioactivity of the stomach and each segment of the small intestine was measured in an automatic $\gamma$ counter (1470 Wizard, Pharmacia, Turku, Finland). Gastric emptying was expressed by determining the amount of labelled chromium contained in the small intestine, 15 min after intubation, as a percentage of the initial amount received (Chen *et al*., 1997; Holzer, 1985). GI transit was assessed by analysing the geometric centre of distribution of the radioactivity within the 10 equal segments (Miller *et al*., 1981; Chen *et al*., 1997). The geometric centre was calculated by summation of % of radioactivity measured in each segment multiplied by the segment number.

### *Processing of plasma*

After the rats were decapitated, blood samples were collected and mixed with EDTA (1 mg $ml^{-1}$ of blood) and aprotinin (500 kiu $ml^{-1}$ of blood). Plasma was immediately obtained by centrifugation at 1000 x *g* for 30 min at 4°C and used for measurement of plasma CCK. The plasma samples were acidified with an equal volume of 1% trifluoroacetic acid (TFA, Buffer A) and then centrifuged at 6000 x *g* for 20 min at 4°C. A SEP-PAK $C_{18}$ cartridge (Waters Associates, Milford, MA, U.S.A.) was equilibrated by 60% acetonitrile in 1% TFA (1 ml, Buffer B) and followed by Buffer A (3 ml, three times). The plasma solution was loaded onto the pretreated $C_{18}$ cartridge. After application of plasma, the cartridge was slowly washed with Buffer A (3 ml, twice) and the peptide slowly eluted with 3 ml Buffer B. The eluant was collected and evaporated in a Speed Vac concentrator (Salvant Instruments, Farmingdale, NY, U.S.A.). The dried samples were maintained at −70°C and subsequently reconstituted with an assay buffer before radioimmunoassay (Hwu *et al*., 1992).

### *Chemical analysis*

CCK concentration in the plasma samples was measured by the RIA kit purchased from Peninsula Laboratories, Belmont (CA, U.S.A.).

### *Drugs*

Chemicals used in the study included: amphetamine (0.75–3 mg $kg^{-1}$), EDTA (1 mg $ml^{-1}$ of blood), aprotinin (500 kiu $ml^{-1}$ of blood) and trifluoroacetic acid (TFA, 1%) were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Proglumide sodium, lorglumide sodium and PD 135,158 (N-methyl-D-glucamine) were purchased from Research Biochemicals International Company (Natick, MA, U.S.A.). Acetonitrile (60% in 1% TFA) was purchased from Wako Chemical Company (Japan). $Na_2{}^{51}CrO_4$ (0.5 $\mu$Ci $ml^{-1}$) was purchased from DuPont NEN Research Products (Boston, MA, U.S.A.).

### *Statistical analysis*

All data are expressed as mean ± s.e. mean. The treatment means were tested for homogeneity using one-way analysis of variance, and the significance of any difference between specific means was tested using Duncan's multiple range test (Steel &

Torrie, 1960). A difference between two means was considered to be statistically significant when $P$ was less than 0.05.

## Results

### *Acute and chronic effects of amphetamine on gastric emptying and intestinal transit*

Both acute (3 mg $kg^{-1}$, i.p.) and chronic (1 mg $kg^{-1}$ $day^{-1}$ for 10 days) pretreatment with amphetamine inhibited the gastric emptying ($24.5 \pm 5.2\%$ and $49.3 \pm 4.8\%$, $n=7-8$, *versus* control group $66.1 \pm 4.8$ %, $n=8$, $P<0.01$) (Figure 1a). Acute administration of amphetamine also significantly inhibited the geometric centre ($2.21 \pm 0.14$, $n=8$, *versus* control group $3.27 \pm 0.22$, $n=8$, $P<0.01$) (Figure 1b). On the other hand, chronic pretreatment of amphetamine did not alter the geometric centre ($3.31 \pm 0.12$, $n=7$) (Figure 1b).

### *Acute effects of different doses of amphetamine on gastric emptying and intestinal transit*

Figure 2a shows the dose-response effect of acute administration of amphetamine on gastric emptying in male rats. Amphetamine at doses of 0.75, 1.5 and 3.0 mg $kg^{-1}$ all significantly ($P<0.01$) inhibited gastric emptying ($31.3 \pm 6.2$, $26.4 \pm 4.0$ and $20.2 \pm 4.0\%$, $n=7-10$) as compared with controls ($67.2 \pm 2.8\%$, $n=10$).

The acute effect of different doses of amphetamine on intestinal transit is illustrated in Figure 2b. Although the

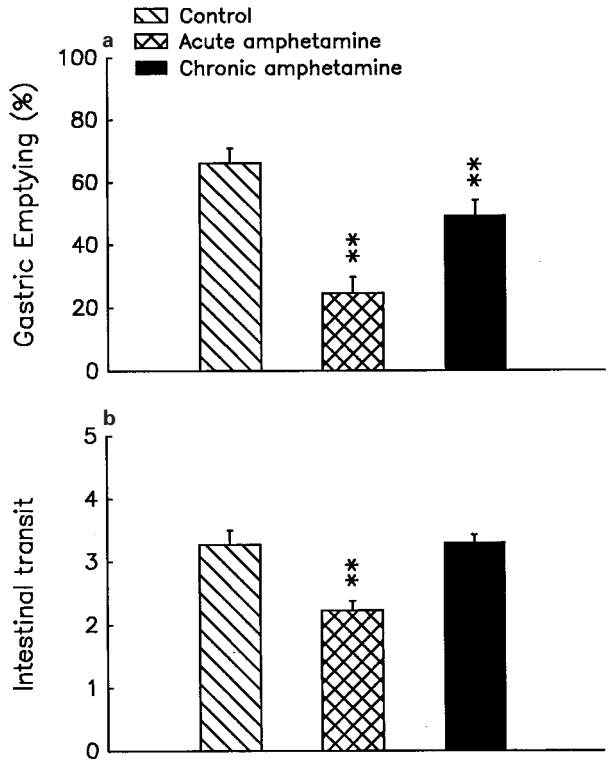


**Figure 1** Acute and chronic effects of amphetamine on gastric emptying (a) and intestinal transit (b) in male rats. Animals received i.p. injections of normal saline (control $n=8$) or 1 mg $kg^{-1}$ $day^{-1}$ amphetamine (chronic; $n=7$) for ten days. On the experimental day, animals in the acute group received an i.p. injection of 3 mg $kg^{-1}$ amphetamine (acute, $n=8$). Other animals were injected i.p. with normal saline 30 min before gastric intubation of $^{51}Cr$ (0.5 $\mu$Ci $ml^{-1}$) and charcoal (10%). Fifteen minutes later, rats were decapitated. The gastric emptying was determined by measuring the amount of labelled chromium contained in the small intestine as a percentage of initial amount received. Intestinal transit, expressed as the geometric centre, was calculated by determining distribution of the radiolabelled marker in the control group, acute group and chronic group. Each column represents the mean $\pm$ s.e. mean. $^{**}P<0.01$ compared with control rats.

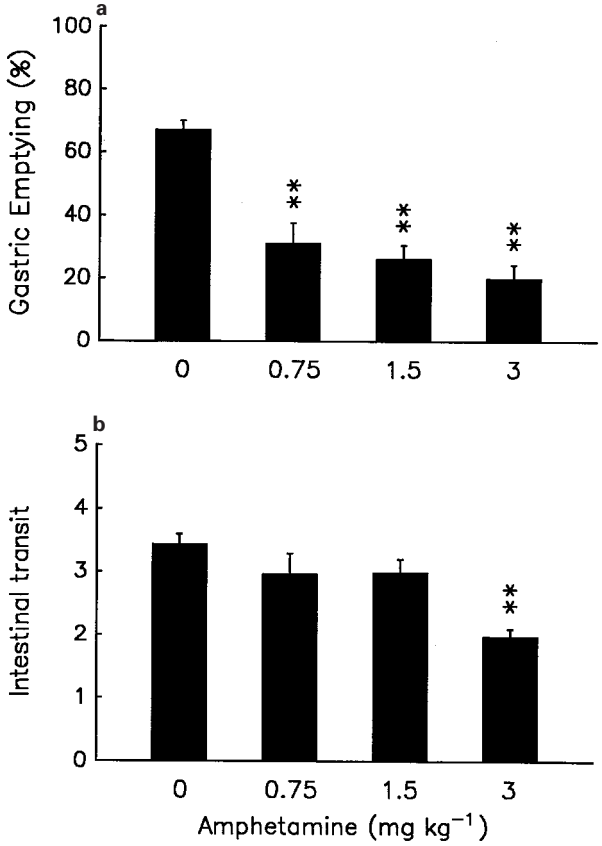


**Figure 2** Effects of different doses of amphetamine ($n=7-10$) on gastric emptying (a) and intestinal transit (b) in male rats. On the experiment day, animals received an i.p. injection of 0, 0.75, 1.5 or 3 mg $kg^{-1}$ amphetamine 30 min before receiving the test meal. Fifteen minutes later, rats were decapitated and blood samples were collected. Please see the legend to Figure 1 for details. Each column represents the mean $\pm$ s.e.mean. $^{**}P<0.01$ compared with control rats.

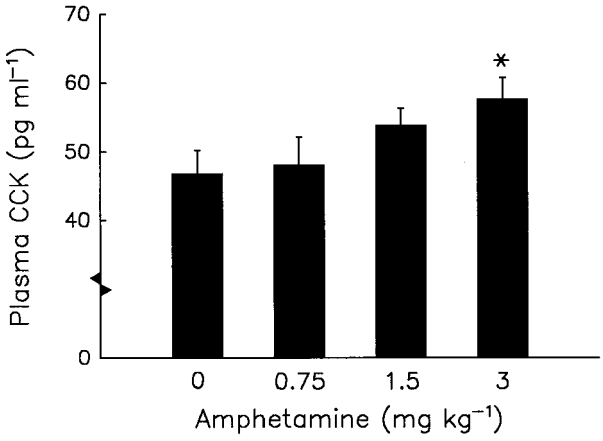


**Figure 3** Effects of different doses of amphetamine ($n=7-8$) on the concentration of plasma CCK in male rats. Please see the legend to Figure 2 for details. Plasma CCK was measured by radioimmunoassay. Each column represents the mean $\pm$ s.e. mean. $^{*}P<0.05$ compared with control rats.

geometric centre was reduced by amphetamine at doses of 0.75, 1.5 and 3.0 mg $kg^{-1}$, only the value ($1.98 \pm 0.11$, $n=10$) obtained at a dose of 3.0 mg $kg^{-1}$ amphetamine was significantly ($P<0.01$) lower than the control value ($3.45 \pm 0.15$, $n=10$). The other two lower doses of amphetamine had no significant effect on the geometric centre.

### *Acute effects of different doses of amphetamine on CCK plasma concentrations*

Plasma CCK was increased dose-dependently by acute administration of different doses of amphetamine (Figure 3). However, only the high dose (3.0 mg $kg^{-1}$) of amphetamine resulted in a significantly greater ($P<0.05$) level of plasma CCK ($57.6 \pm 3.0$ pg $ml^{-1}$, $n=7$) compared to the control value ($46.8 \pm 3.4$ pg $ml^{-1}$, $n=7$) (Figure 3). The other two lower doses of amphetamine had no significant effect on the level of plasma CCK ($48.2 \pm 4.0$ and $53.8 \pm 2.4$ pg $ml^{-1}$, $n=8$) (Figure 3).

### *Effects of proglumide on the inhibition of gastric emptying and intestinal transit by amphetamine*

Proglumide (10 mg $kg^{-1}$) had no effect on gastric emptying ($65.8 \pm 7.7\%$, $n=8$, *versus* saline group $66.9 \pm 5.4\%$, $n=7$) (Figure 4a) and intestinal transit (geometric centre values: $3.30 \pm 0.25$, $n=8$, *versus* saline group $3.39 \pm 0.14$, $n=7$) (Figure 4b) in male rats. Pretreatment of proglumide significantly attenuated ($48.4 \pm 4.9\%$, $n=7$) ($P<0.05$) the inhibition of gastric emptying by amphetamine ($26.3 \pm 5.2\%$, $n=8$) (Figure 4a) and prevented ($3.33 \pm 0.59$, $n=7$) ($P<0.05$) the inhibition of intestinal transit by amphetamine ($2.24 \pm 0.13$, $n=8$) (Figure 4b).

### *Effects of different doses of proglumide on the inhibition of gastric emptying and intestinal transit by amphetamine*

Low dose of proglumide (3 mg $kg^{-1}$) mildly attenuated the effect of amphetamine on gastric emptying ($41.6 \pm 5.5\%$, $n=6$, *versus* amphetamine group $26.2 \pm 1.7\%$, $n=7$) (Figure 5a) and intestinal transit (geometric centre values: $2.20 \pm 0.20$, $n=6$, *versus* amphetamine group $1.83 \pm 0.17$, $n=7$) (Figure 5b). Pretreatment of another two higher doses of proglumide significantly prevented ($P<0.05$) the amphetamine-induced inhibition of gastric emptying ($49.7 \pm 8.2$ and $47.9 \pm 6.1\%$, $n=6$, *versus* amphetamine group $26.2 \pm 1.7\%$, $n=7$) (Figure 5a) and intestinal transit ($2.57 \pm 0.29$ and $2.51 \pm 0.17$, $n=6$, *versus* amphetamine group $1.83 \pm 0.17$, $n=7$) (Figure 5b).

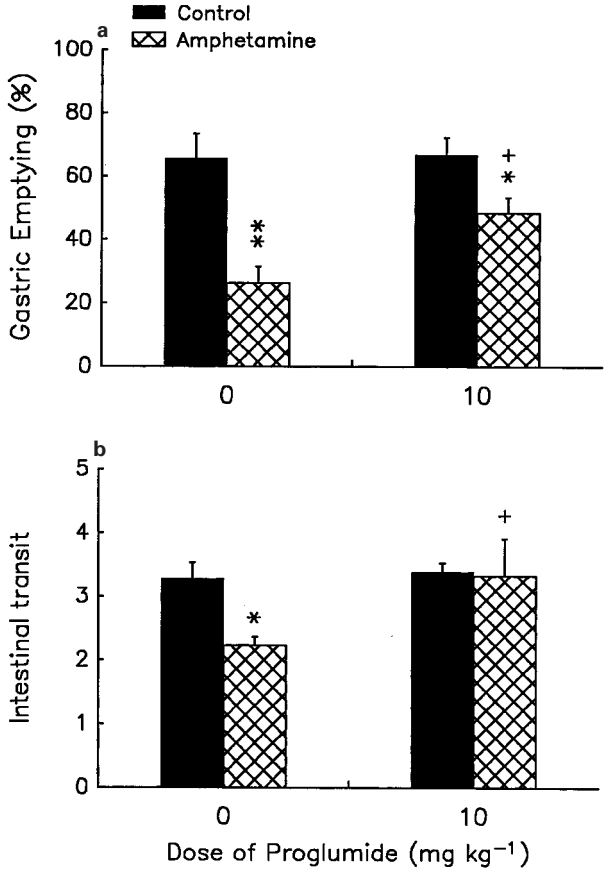


**Figure 4** Effects of proglumide on the suppression of gastric emptying (a) and intestinal transit (b) by amphetamine in male rats. On the experimental day, animals received an i.p. injection of normal saline (control, $n=7-8$) or amphetamine (3 mg $kg^{-1}$; $n=7-8$) 10 min after i.p. injection of normal saline or proglumide (10 mg $kg^{-1}$). Thirty minutes later, all rats were given the test meal. Please see the legend to Figure 1 for details. Each column represents the mean ± s.e. mean. $^{*}P<0.05$ and $^{**}P<0.01$ compared with control rats, respectively. $^{+}P<0.05$ compared with saline plus amphetamine group.

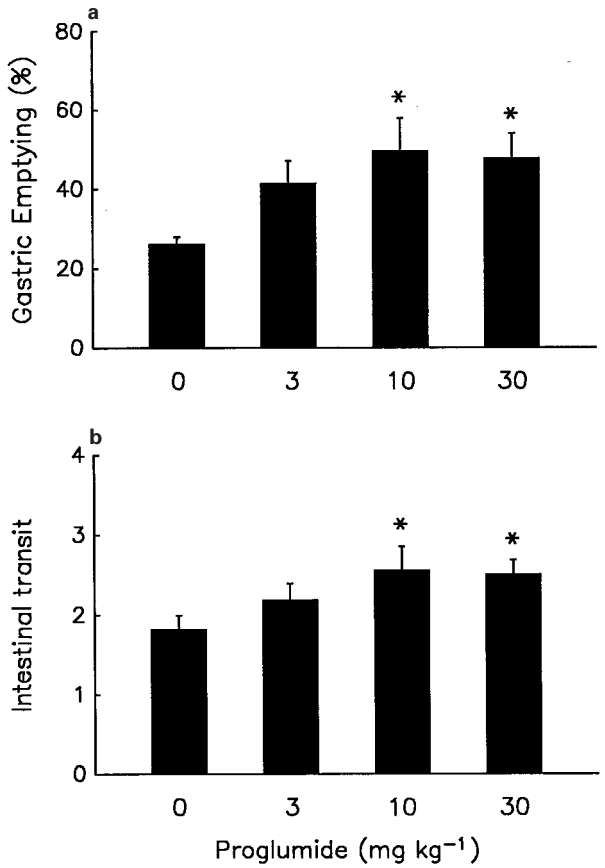


**Figure 5** Effects of different doses of proglumide ($n=6-7$) on the suppression of gastric emptying (a) and intestinal transit (b) by amphetamine in male rats. Male rats received an i.p. injection of 0, 3, 10 or 30 mg $kg^{-1}$ proglumide and 10 min later an i.p. injection of amphetamine (3 mg $kg^{-1}$). Thirty minutes after injection of amphetamine, rats were given the test meal. Please see the legend to Figure 1 for details. Each column represents the mean ± s.e. mean. $^{*}P<0.05$ compared with the group given 0 mg $kg^{-1}$ proglumide.

*Effects of lorglumide on the inhibition of gastric emptying and intestinal transit by amphetamine*

Pretreatment with the higher dose (10 mg $kg^{-1}$) of lorglumide significantly prevented ($P<0.01$) the amphetamine-induced inhibition of gastric emptying (49.2±6.9%, $n=7$, *versus* saline plus amphetamine group 21.7±5.4%, $n=7$) (Figure 6a). Lorglumide (5 mg $kg^{-1}$) mildly attenuated the effect of amphetamine on gastric emptying (34.3±4.6%, $n=7$, *versus* saline plus amphetamine group 21.7±5.4%, $n=7$) (Figure 6a). Pretreatment with 5 or 10 mg $kg^{-1}$ lorglumide mildly attenuated the inhibitory effect of amphetamine on intestinal transit (geometric centre values: 2.36±0.11 and 2.68±0.35, $n=7$, *versus* saline plus amphetamine group 2.07±0.26, $n=7$) (Figure 6b).

*Effects of PD 135,158 on the inhibition of gastric emptying and intestinal transit by amphetamine*

Pretreatment with 5 or 10 mg $kg^{-1}$ PD 135,158 did not reverse the amphetamine-induced inhibition of gastric emptying (33.0±4.3 and 29.6±5.3 %, $n=7$, *versus* saline plus amphetamine group 28.4±5.8%, $n=7$) (Figure 7a). Thus, administration of amphetamine resulted in a significant inhibition ($P<0.01$) of gastric emptying, regardless of the pretreatment with PD 135,158 as compared with controls (69.5±6.1%, $n=8$). Pretreatment with 5 or 10 mg $kg^{-1}$ PD 135,158 mildly attenuated the inhibitory effect of amphetamine on intestinal transit (2.64±0.25 and 3.21±0.40, $n=7$, *versus* saline plus amphetamine group 2.29±0.35, $n=7$) (Figure 7b).

## Discussion

The present results demonstrate that (1) both acute and chronic administration of amphetamine inhibits gastric emptying in male rats; (2) intestinal transit is decreased by the acute but not chronic administration of amphetamine; (3) administration of amphetamine increases plasma CCK levels; (4) proglumide partially attenuated the amphetamine-induced inhibition of gastric emptying and intestinal transit; (5) the selective $CCK_A$ receptor antagonist, lorglumide, partially blocked amphetamine-induced inhibition of gastric emptying in male rats, but the selective $CCK_B$ receptor antagonist, PD 135,158, did not reverse the inhibitory effect of amphetamine on gastric emptying; and (6) $CCK_A$ and $CCK_B$ receptor

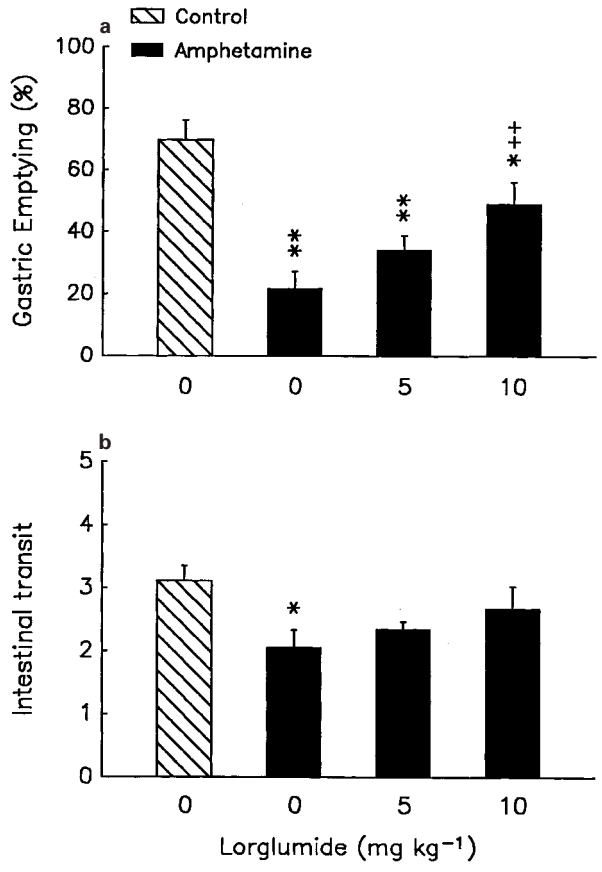


**Figure 6** Effects of lorglumide on the inhibition of gastric emptying (a) and intestinal transit (b) by amphetamine in male rats. On the experiment day, animals received an i.p. injection of 0, 5, or 10 mg $kg^{-1}$ lorglumide and 10 min later an i.p. injection of normal saline (control, $n=7$) or amphetamine (3 mg $kg^{-1}$, $n=7$). Thirty minutes after injection of saline or amphetamine, rats were given the test meal. Please see the legend to Figure 1 for details. Each column represents the mean±s.e. mean. $^{*}P<0.05$ and $^{**}P<0.01$ compared with saline-injected rats, respectively. $^{++}P<0.01$ compared with the group given 0 mg $kg^{-1}$ lorglumide.

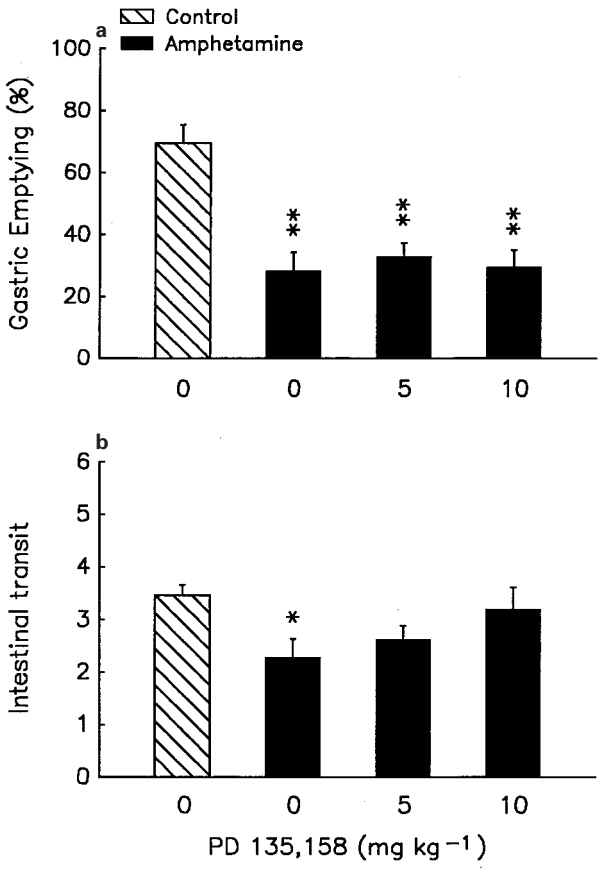


**Figure 7** Effects of PD 135,158 on the inhibition of gastric emptying (a) and intestinal transit (b) by amphetamine in male rats. On the experiment day, animals received an i.p. injection of 0, 5 or 10 mg $kg^{-1}$ PD 135,158 and 10 min later an i.p. injection of normal saline (control, $n=7-8$) or amphetamine (3 mg $kg^{-1}$; $n=7-8$). Thirty minutes after the injection of saline or amphetamine, rats were given the test meal. Please see the legend to Figure 1 for details. Each column represents the mean±s.e. mean. $^{*}P<0.05$ and $^{**}P<0.01$ compared with saline-injected rats, respectively.

antagonists partially attenuated the inhibitory effect of amphetamine on intestinal transit in male rats.

Administration of amphetamine has been shown to cause or facilitate a loss of body weight in obese and non-obese persons; loss of weight was found to be primarily due to an inhibition in the intake of food (Colton *et al*., 1943; Tainter, 1944; Harris *et al*., 1947; Bray, 1993). Complex mechanisms in regulating food intake have been reported (Wellman, 1990a). Although food intake was not measured in this study, it is well established that food intake is decreased by amphetamine. A dose of amphetamine (3 mg $kg^{-1}$, i.p.) is enough to suppress feeding and does not induce an aversive response in the taste reactivity test (Wellman & Peters, 1980). It is well known that the anorexic action of amphetamine is linked to the activation of dopamine receptors and/or $\beta$-adrenoceptors by amphetamine within the lateral hypothalamus (Booth, 1968; Baze, 1974; Leibowitz, 1975). Amphetamine anorexia is prevented by pretreatment with dopamine antagonists (Baze, 1974; Wellman, 1990b).

It has been documented that the stomach contains nutrient receptors that serve to signal the presence of food in the stomach and thereby inhibit further food intake (Deutsch, 1983). Thus the signals generated from the stomach and/or gut may play an important role in the regulation of food intake. The present study demonstrated that both acute and chronic administration of amphetamine inhibit gastric emptying in male rats. These results are compatible with results obtained in man (VanLiere & Sleeth, 1939) and rodents (Bridges *et al*., 1975). Gastric emptying is thus an important factor in regulation of feeding. The anorexic action of amphetamine may reflect, in part, an inhibitory effect on gastric emptying (Davies *et al*., 1983). Inhibition of gastric emptying by amphetamine has been suggested to result from retention of nutrients within the stomach, from which arises either a prolonged inhibitory nutrient signal to the brain or an intensified signal generated by entry of nutrient into the stomach (Wellman, 1990a). The suppression of appetite is then due, in part, to an inhibition of gastric emptying and GI transit.

Dietary administration of amphetamine (up to 5 mg $kg^{-1}$) has no effect on transit time in rats, although amphetamine administered via the subcutaneous route decreases transit time (Tainter, 1944). In the present study, although the high dose (3 mg $kg^{-1}$) of amphetamine acutely administered via the intraperitoneal route also decreased intestinal transit, the effect of lower doses of amphetamine had no significant effect. It has been reported that small intestine emptying is influenced by the amount of test meal present (Purdon & Bass, 1973). Therefore, the decrease in intestinal transit by the higher dose of amphetamine may result in part from smaller volumes entering the small bowel following inhibited gastric emptying. The fact that the lower doses of amphetamine had no effect on intestinal transit may be due to an insufficient dose of amphetamine or larger volumes entering the small bowel.

It is known that gut hormone CCK delays gastric emptying in both animals and man. (Chey *et al*., 1970; Debas *et al*., 1975; Anika, 1982; Mangel & Koegel, 1984; Jin *et al*., 1994). Exogenous CCK decreases food intake in adult rats (Gibbs *et al*., 1973) and monkeys (Gibbs *et al*., 1976). Endogenous secretion of CCK by L-phenylalanine reduces food intake in man (Ballinger & Clark, 1994). The investigations reveal that CCK suppresses food intake by inhibiting gastric emptying (Moran & McHugh, 1982). In the present study, we found that plasma CCK levels were increased and gastric emptying was decreased by amphetamine in a dose-dependent manner. Therefore, we suggest that the inhibition of GI transit by amphetamine is due in part to CCK secretion.

Endogenous and exogenous CCK delays gastric emptying of liquids through stimulation of $CCK_A$ receptors (Beglinger, 1994; Moran *et al*., 1994; Varga & Scarpignato, 1996). It has been suggested that CCK produces a decrease in the rate of gastric emptying by causing contraction of pyloric sphincter and $CCK_A$ receptor antagonism is known to reverse this effect (Murphy *et al*., 1987; Margolis *et al*., 1989). In man, $CCK_A$ receptors are involved in the modulation of gastric sensory and motor responses to gastric distension and duodenal lipid (Feinle *et al*., 1996). In rats, both $CCK_A$ and $CCK_B$ receptor mRNA has been detected in stomach (Monstein *et al*., 1996). In our studies CCK receptor antagonists, proglumide, lorglumide and PD 135,158, were employed to evaluate if the inhibition of gastric emptying by amphetamine is due to the action of CCK. We found that proglumide, a selective CCK receptor antagonist that binds preferentially to $CCK_A$ receptors (Hahne *et al*., 1981) partially prevented the inhibition of gastric emptying by amphetamine. Lorglumide is more potent than proglumide in blocking $CCK_A$ receptors (Makovec *et al*., 1986). Lorglumide also partially blocked amphetamine-induced inhibition of gastric emptying in male rats. Nevertheless, lorglumide did not completely restore the gastric emptying to that of the control value. Since multiple factors affect gastric emptying, it is likely that other factors may be involved in amphetamine-induced inhibition of gastric emptying. The selective $CCK_B$ receptor antagonist, PD 135,158 (Hughes *et al*., 1990), did not reverse the effect of amphetamine on gastric emptying. We therefore suggest that the decreased gastric emptying induced by amphetamine administration may, in part, be mediated by the hypersecretion of endogenous CCK. CCK delays gastric emptying through activation of $CCK_A$ receptors rather than $CCK_B$ receptors.

One of the physiological actions of CCK in the gastrointestinal tract is to regulate intestinal motility. Little is known about the mechanism by which CCK regulates small intestinal transit after feeding. In man, endogenous CCK is a major stimulator, regulator of antro-pyloroduodenal motility in the intestinal phase and has a direct effect on smooth muscle CCK receptors in the duodenum (Katschinski *et al*., 1996). Stimulation of duodenal contractile activity by CCK would retard gastric emptying, whereas inhibition of duodenal motility would be a further mechanism for $CCK_A$ receptor antagonists to accelerate gastric emptying (Haba & Sarna, 1993). Endogenous CCK is involved in the regulation of migrating myoelectric complexes (MMCs) through stimulation of peripheral $CCK_B$ receptors (Rodriguez-Membrilla & Vergara, 1997). Exogenous polyamines disrupt intestinal MMCs through the release of CCK acting at $CCK_A$ and $CCK_B$ receptors (Fioramonti *et al*., 1994). The present results indicate that proglumide partially attenuated the amphetamine-induced inhibition of intestinal transit. Moreover, pretreatment with lorglumide or PD 135,158 produced a similar result to proglumide. These findings suggest that the decreased intestinal transit induced by amphetamine administration may, in part, be mediated by the hypersecretion of endogenous CCK acting at both $CCK_A$ and $CCK_B$ receptors.

In summary, the present investigations demonstrate that administration of amphetamine in male rats causes a slower gastric emptying and intestinal transit, which correlate with an increase in CCK plasma concentration. Amphetamine-induced inhibition of gastric emptying was partially attenuated by $CCK_A$ but not $CCK_B$ receptor antagonism. However, amphetamine-induced inhibition of intestinal transit was partially attenuated by either $CCK_A$ or $CCK_B$

receptor antagonism. These results suggest that amphetamine inhibition of gastric emptying and intestinal transit is in part mediated by the hypersecretion of endogenous CCK.

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