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The Roles of Dopamine Receptor and Adrenoreceptor on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine in Male Rats

Wei-Ju Huang[1], Eileen Jea Chien[2], Jiun-Yih Yeh[3], Jiann-Jong Chen[4], Yu-Chung Chiao[2], Paulus S. Wang[2], Full-Young Chang[3,*], and Ming-Luen Doong[2,*]

[1]*Department of Nursing, Hsin-Sheng College of Medical Care and Management, Taoyuan 32544*
[2]*Department of Physiology, National Yang-Ming University, Taipei 11221*
[3]*Division of Gastroenterology, Department of Medicine, Veterans General Hospital-Taipei Taipei 11217*
and
[4]*Cardinal Tien College of Healthcare and Management, New Taipei City 23143*
Taiwan, Republic of China

Abstract

Amphetamine, a central nervous stimulant, acts on the central nervous system (CNS) by increasing levels of norepinephrine, serotonin and dopamine (DA) in the brain. It stimulates the colonic motility and gastrointestinal (GI) function *via* the brain-gut connections as well as diminishes appetite to control bodyweight, but the mechanism between amphetamine and GI motility was unclear. We investigated the role of DA receptor and adrenoreceptor in the inhibitory effects of amphetamine on GI motility in male rats. After fasting overnight, the male rats received an intraperitoneal (i.p.) injection of amphetamine (3 mg/ml/kg) 15 min after i.p. injection of different antagonists for dopamine receptors and adrenoreceptors. Gastric emptying and intestinal transit were assessed 15 min after intragastric instillation of a test meal containing radioactive $Na_2{}^{51}CrO_4$ and 10% charcoal. Haloperidol, a DA receptor antagonist, acting exactly as the selective D_2 receptor antagonist, eticlopride, completely prevented amphetamine-induced inhibition of gastric emptying and intestinal transit in male rats. The selective D_1 receptor antagonist, SCH 23390, significantly attenuated the amphetamine-induced inhibition of gastric emptying and intestinal transit in male rats. Both selective and nonselective α adrenoreceptor antagonist, phentolamine, prazosin and yohimbine, did not reverse the inhibitory effects of amphetamine on gastric emptying and intestinal transit. Propanolol, a β adrenoreceptor antagonist, partially attenuated the amphetamine-induced inhibition of gastric emptying but did not significantly attenuate intestinal transit. These results suggest that amphetamine-induced inhibition of gastric emptying and intestinal transit is mediated by an action on dopaminergic mechanism and adrenoreceptor with little involvement. These findings may clarify the influence of central nervous stimulant on GI tract and may provide the appropriate medication in CNS.

Key Words: amphetamine, gastric emptying, intestinal transit, geometric center, dopamine receptor antagonists, adrenoreceptor antagonists

Corresponding author: Ming-Luen Doong, Ph.D., Department and Institute of Physiology, National Yang-Ming University, Shih-Pai, Taipei 11221, Taiwan, Republic of China. Tel: +886-2-28267283, Fax: +886-2-28264049, E-mail: doong@ym.edu.tw
*These authors contributed equally to this work.
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Introduction

Amphetamine is classified as a stimulant of the central nervous system (CNS) and is also a dopamine (DA) agonist which promotes DA and norepinepherine (NE) releases from the end of neuron synapse and inhibits the reuptake in neuron synapse (2, 17). It has been shown that amphetamine can reduce appetite and food intake (3, 6, 14) *via* DA receptors and β-adrenoreceptors (4, 18). Besides, amphetamine acts on the sympathetic nervous system, the activity of which is associated with energy balance, metabolic syndrome and food intake (16). DA regulates food intake by modulating food reward and motivation (12, 25) without crossing the blood-brain barrier. Pretreatment of DA antagonist attenuates appetite reduction of amphetamine *via* the inhibition of gastric emptying (33). On the other hand, it has been found that stimulation of α_1-adrenoceptors suppresses food and water intake (20). The β-adrenoceptor has been suggested to be involved in regulation of gut motility and visceral algesia (8). It is also believed that food ingestion is mediated in part by sympathetic nervous system activation and consequent β-adrenergic receptor stimulation of metabolism (28). Our previous study showed that acute treatment with amphetamine inhibited the gastric emptying and intestinal transit in male rats accompanied with the increased plasma cholecystokinin (CCK). CCK, a gastrointestinal hormones, is produced in discrete endocrine cells that line the mucosa of the small intestine hormone and is responsible for gallbladder contraction and pancreatic enzyme secretion. CCK probably functions as a neurotransmitter found in the central nervous system and the peripheral nerves that innervate the intestine (34). 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.

The inhibitory effects of gastric emptying and intestinal transit are partly due to the increase of intrinsic CCK secretion and its receptors. Moreover, studies have proved that amphetamine can reduce food intake and appetite *via* DA receptors and β-adrenoreceptors (4, 18). However, signals generated from the gastrointestinal (GI) tract may play an important role in regulation of food intake. It is interesting to determine which types of DA receptors and adrenoceptors are involved in regulation of amphetamine on GI motility. Therefore, the aim of the present study was to investigate the role of DA receptor and adrenoreceptor in the inhibitory effects of amphetamine on GI motility. Haloperidol (a DA receptor antagonist), SCH 23390 (a D_1 receptor antagonist), eticlopride (a D_2 receptor antagonist), phentolamine (an α adrenoceptor antagonist), propranolol (a β adrenoceptor antagonist), prazosin (an α_1 adrenoceptor antagonist) and yohimbine (an α_2 adrenoceptor antagonist) (5, 9, 27) were used to clarify which type of DA receptors and adrenoceptors were involved in the inhibition of gastric emptying and intestinal transit in amphetamine-treated male rats.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 250-350 *g* were housed in a temperature (22 ± 1°C) and light (6 a.m. - 8 p.m.) controlled environment and fed with rat chow. Tap water was given *ad libitum*. Animal protocols were approved by the Institutional Animal Care and Use Committee of National Yang-Ming University. All animals received humane 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, R.O.C.

Measurement of Gastric Emptying and Gastrointestinal Transit

In the Experiments, rats were divided into 4 groups and fasted for 20-24 h before the experiment of gastric emptying. On the day of the experiment (around 9 a.m.), conscious rats were intubated *via* a catheter (PE-205, ID: 1.67 mm, OD: 2.42 mm, Clay-Adam, USA) with physiological saline (3 ml/kg) containing $Na_2{}^{51}CrO_4$ (0.5 μCi/ml) 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 min later, the rats were decapitated. The stomach and the attached small intestine were immediately exposed by a laparotomy. After ligating the esophagogastric, gastroduodenal and ileocecal 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 the divided equally into 10 segments. The amounts of radioactivity of the stomach and each segment of the small intestine were measured in an automatic gamma counter (1470 Wizard, Pharmacia, Turku, Finland). Gastric emptying was expressed by determining the amount of labeled chromium contained in the small intestine 15 min after intubation as a percentage of the initial amount received. Intestinal transit was assessed by analyzing the geometric center of distribution of the radioactivity within the 10 equal segments (9). The geometric center was calculated by summation of

percent of radioactivity measured in each segment multiplied by the segment number.

Experimental Procedure

Experiment 1

Rats were divided into four groups and fasted for 20-24 h before use. They received an i.p. injection of vehicle (0.2% acetic acid in 30% ethanol) or haloperidol (a DA receptor antagonist, 2.5 mg/kg) 15 min before an i.p. injection of normal saline or amphetamine (3 mg/kg). Thirty minutes later, rats received the test meal. Fifteen minutes after administration of the liquid meal, rats were decapitated and intestinal transit was measured.

Experiment 2

Rats were fasted for 20-24 h prior to use. They received an i.p. injection of normal saline or one of the selective DA receptor antagonists including SCH 23390 (a D_1 receptor antagonist, 0.2 mg/kg) and eticlopride (a D_2 receptor antagonist, 0.5 mg/kg) at 15 min before an i.p. injection of normal saline or amphetamine (3 mg/kg). Thirty minutes later, rats received the test meal. Fifteen minutes after administration of the liquid meal, rats were decapitated and intestinal transit was measured.

Experiment 3

Rats were divided into four groups and fasted for 20-24 h before use. They received an i.p. injection of normal saline or phentolamine (an α adrenoreceptor antagonist, 5 mg/kg) 15 min before an i.p. injection of normal saline or amphetamine (3 mg/kg). Thirty minutes later, rats received the test meal. Fifteen minutes after administration of the liquid meal, rats were decapitated and intestinal transit was measured.

Experiment 4

Rats were divided into four groups and fasted for 20-24 h before use. They received an i.p. injection of normal saline or one of the selective adrenoreceptor antagonists including prazosin (a α_1 receptor antagonist, 0.5 mg/kg) and yohimbine (a α_2 receptor antagonist, 5 mg/kg) at 15 min before an i.p. injection of normal saline or amphetamine (3 mg/kg). Thirty minutes later, rats received the test meal. Fifteen minutes after administration of the liquid meal, rats were decapitated and intestinal transit was measured.

Experiment 5

Rats were divided into four groups and fasted for 20-24 h before use. They received an i.p. injection of normal saline or propranolol (a β adrenoreceptor antagonist, 1 mg/kg) 15 min before an i.p. injection of normal saline or amphetamine (3 mg/kg). Thirty minutes later, rats received the test meal. Fifteen minutes after administration of the liquid meal, rats were decapitated and intestinal transit was measured.

Drugs

Chemicals used in the study including amphetamine (0.75 - 3 mg/kg), EDTA (1 mg/ml of blood), aprotinin (500 KIU/ml of blood) and trifluoroacetic acid (TFA, 1%) were purchased from Sigma Chemical Company, St. Louis, MO., USA. SCH 23390, eticlopride and yohimbine were purchased from Research Biochemicals International Company, Natick, MA, USA. Haloperidol, phentolamine, propranolol and prazosin were purchased from Sigma. Acetonitrile (60% in 1% TFA) was purchased from Wako Chemical Company, Japan, and $Na_2{}^{51}CrO_4$ (0.5 μCi/ml) was purchased from DuPont NEN Research Products, Boston, MA, USA.

Statistical Analysis

All data were expressed as means ± SEM. 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. A difference between two means was considered to be statistically significant when P was less than 0.05.

Results

Effects of Haloperidol on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine

Haloperidol (2.5 mg/kg) had no effects on gastric emptying (70.7 ± 3.7%, n = 7, versus the saline group 67.7 ± 2.6%, n = 6) (Fig. 1A) and intestinal transit (geometric center values: 3.08 ± 0.16, n = 7, versus the saline group 3.37 ± 0.16, n = 6) (Fig. 1B) in male rats. Pre-treatment of haloperidol completely reversed (68.0 ± 7.3%, n = 7) ($P < 0.01$) the inhibition of gastric emptying by amphetamine (25.8 ± 4.1%, n = 6) (Fig. 1A) and prevented (3.35 ± 0.27, n = 7) ($P < 0.01$) the inhibition of intestinal transit by amphetamine (2.15 ± 0.26, n = 6) (Fig. 1B).

Effects of SCH 23390 on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine

SCH 23390 (0.2 mg/kg) had no effects on gastric

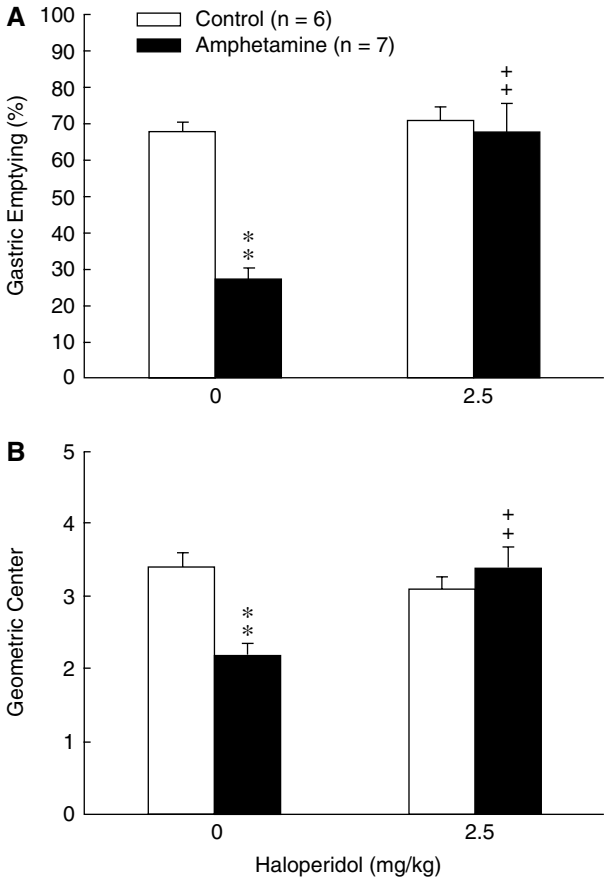

Fig. 1. Effects of haloperidol on the inhibition of gastric emptying (A) and intestinal transit (B) by amphetamine in male rats. On the experiment day, the rats received i.p. injection of normal saline (blank column, n = 6) or amphetamine (3 mg/kg) (solid column, n = 7) 30 min after i.p. injection of vehicle or haloperidol (2.5 mg/kg). Thirty minutes later, all rats were given the test meal containing ^{51}Cr (0.5 μCi/ml) and charcoal (10%). Fifteen minutes later, rats were decapitated. The gastric emptying was determined by measuring the amount of labeled chromium contained in the small intestine as a percentage of the initial amount received. The geometric center was calculated by determining distribution of the radiolabeled marker in control group, acute group and chronic group. Each column represents the means ± SEM. $^{**}P < 0.01$ compared with saline-injected rats. $^{++}P < 0.01$ compared with saline plus amphetamine group.

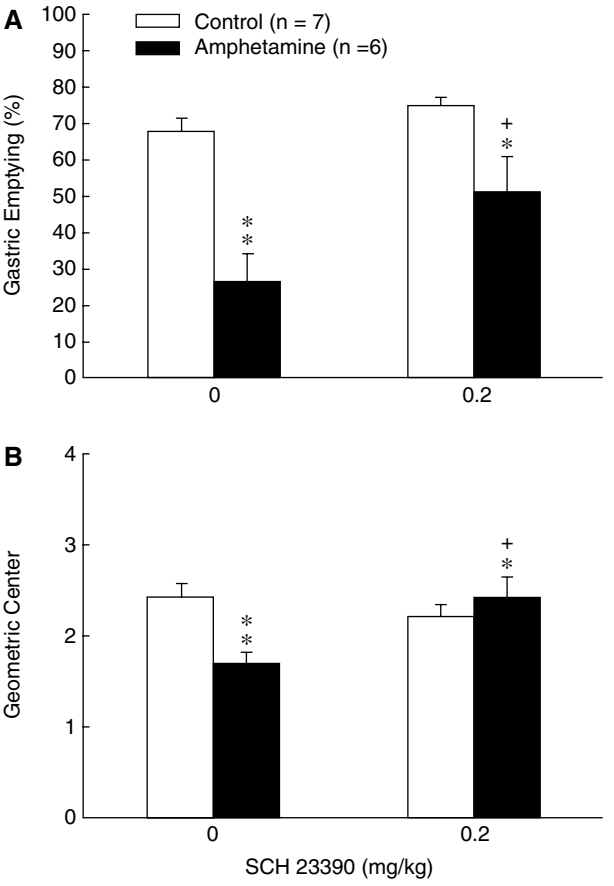

Fig. 2. Effects of SCH 23390 on the inhibition of gastric emptying (A) and intestinal transit (B) by amphetamine in male rats. On the experiment day, animals received i.p. injection of normal saline (blank column, n = 7) or amphetamine (3 mg/kg) (solid column, n = 6) 15 min after i.p. injection of normal saline or SCH 23390 (0.2 mg/kg). Thirty minutes later, all rats were given the test meal. Fifteen minutes later, rats were decapitated. See the legend to Fig. 1 for further details. Each column represents the means ± SEM. $^{*, **}P < 0.05$ and $P < 0.01$ compared with saline-injected rats, respectively. $^{+}P < 0.05$ compared with saline plus amphetamine group.

emptying (74.9 ± 2.6 %, n = 7, versus the saline group 67.6 ± 3.7%, n = 7) (Fig. 2A) and intestinal transit (geometric center values: 2.93 ± 0.09, n = 7, versus the saline group 2.84 ± 0.10, n = 7) (Fig. 2B) in male rats. Pre-treatment of SCH 23390 significantly attenuated (51.3 ± 9.9%, n = 6) ($P < 0.05$) the inhibition of gastric emptying by amphetamine (26.9 ± 7.5%, n = 6) (Fig. 2A) and mildly prevented (2.47 ± 0.19, n = 6) ($P < 0.05$) the inhibition of intestinal transit by amphetamine (2.01 ± 0.15, n = 6) (Fig. 2B).

Effects of Eticlopride on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine

Eticlopride (0.5 mg/kg) had no effects on gastric emptying (64.0 ± 4.3%, n = 7, versus the saline group 64.2 ± 5.7%, n = 8) (Fig. 3A) and intestinal transit (geometric center values: 3.48 ± 0.15, n = 7, versus the saline group 3.53 ± 0.14, n = 8) (Fig. 3B) in male rats. Pre-treatment of eticlopride completely blocked (64.1 ± 5.1%, n = 7) ($P < 0.01$) the inhibition of gastric emptying by amphetamine (39.9 ± 5.0 %, n = 8) (Fig. 3A) and prevented (3.26 ± 0.22, n = 7) ($P < 0.01$)

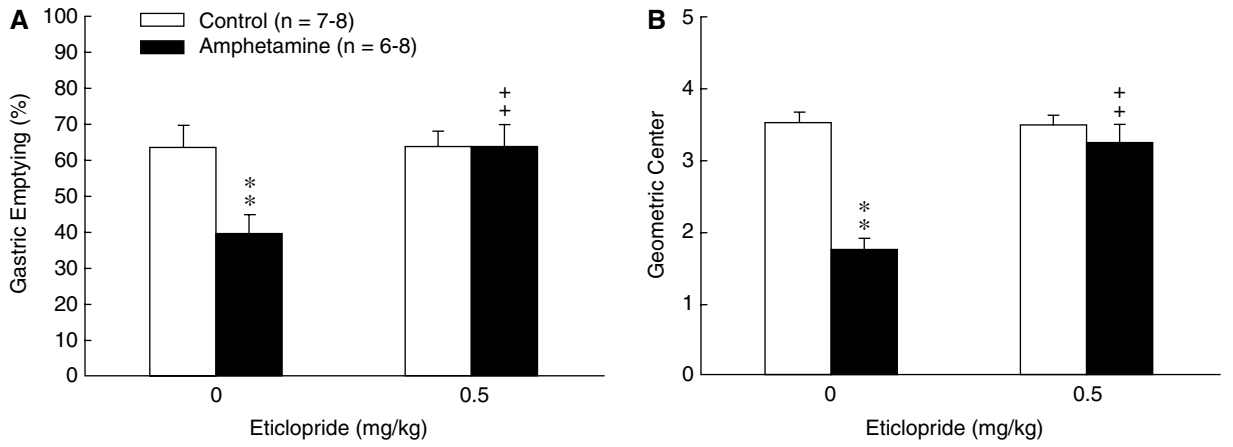

Fig. 3. Effects of eticlopride on the inhibition of gastric emptying (A) and intestinal transit (B) by amphetamine in male rats. On the experiment day, animals received i.p. injection of normal saline (blank column, n = 7-8) or amphetamine (3 mg/kg) (solid column, n = 6-8) 15 min after i.p. injection of normal saline or eticlopride (0.5 mg/kg). Thirty minutes later, all rats were given the test meal. Fifteen minutes later, rats were decapitated. See the legend to Fig. 1 for further details. Each column represents the means ± SEM. $^{**}P < 0.01$ compared with saline-injected rats. $^{++}P < 0.01$ compared with saline plus amphetamine group.

Table 1. Effects of different α-adrenoreceptor antagonists influence the inhibition of gastric emptying and intestinal transit by amphetamine in male rats

Treatment	n	Gastric Emptying (%)	Intestinal Transit
α adrenoreceptor antagonist (Phentolamine)			
Saline	8	66.3 ± 3.6	3.19 ± 0.16
Phentolamine	8	63.8 ± 3.1	3.27 ± 0.24
Amphetamine	8	18.6 ± 6.5**	1.92 ± 0.32**
Phentolamine + Amphetamine	8	20.5 ± 6.5**	2.07 ± 0.24**
Selective α_1 adrenoreceptor antagonist (Prazosin)			
Saline	7	68.2 ± 3.8	3.43 ± 0.17
Prazosin	7	66.9 ± 7.7	3.22 ± 0.33
Amphetamine	7	21.7 ± 7.6**	2.25 ± 0.21**
Prazosin + Amphetamine	7	22.8 ± 7.0**	2.07 ± 0.21**
Selective α_2 adrenoreceptor antagonist (Yohimbine)			
Saline	8	66.2 ± 1.7	3.30 ± 0.19
Yohimbine	7	66.7 ± 6.4	3.13 ± 0.16
Amphetamine	8	21.9 ± 5.0**	1.82 ± 0.21**
Yohimbine + Amphetamine	6	31.0 ± 5.0**	1.68 ± 0.07**

Each data represents the means ± SEM. $^{**}P < 0.01$ compared with saline-injected rats.

the inhibition of intestinal transit by amphetamine (1.77 ± 0.14, n = 8) (Fig. 3B).

Effects of Phentolamine on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine

Phentolamine (5 mg/kg) had no effects on gastric emptying (63.8 ± 3.1%, n = 8, versus the saline group 66.3 ± 3.6%, n = 8) (Table 1) and intestinal transit (geometric center values: 3.27 ± 0.24, n = 8, versus the saline group 3.19 ± 0.16, n = 8) (Table 1) in male rats. Pre-treatment of phentolamine did not reverse the inhibition of gastric emptying (20.5 ± 6.5%, n = 8 versus the amphetamine group 18.6 ± 6.5%, n = 8) (Table 1) and intestinal transit (geometric center values: 2.07 ± 0.24, n = 8, versus the amphetamine group 1.92 ± 0.32, n = 8) (Table 1) by amphetamine.

Effects of Prazosin on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine

Prazosin (0.5 mg/kg) had no effects on gastric

emptying (66.9 ± 7.7%, n = 7, versus the saline group 68.2 ± 3.8%, n = 7) (Table 1) and intestinal transit (geometric center values: 3.22 ± 0.33, n = 7, versus the saline group 3.43 ± 0.17, n = 7) (Table 1) in male rats. Pre-treatment of prazosin did not reverse the inhibition of gastric emptying (22.8 ± 7.0%, n = 7 versus the amphetamine group 21.7 ± 7.6%, n = 7) (Table 1) and intestinal transit (geometric center values: 2.07 ± 0.21, n = 7, versus the amphetamine group 2.25 ± 0.21, n = 7) (Table 1) by amphetamine.

Effects of Yohimbine on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine

Yohimbine (5 mg/kg) had no effects on gastric emptying (66.7 ± 6.4%, n = 8, versus the saline group 66.2 ± 1.7%, n = 7) (Table 1) and intestinal transit (geometric center values: 3.13 ± 0.16, n = 8, versus the saline group 3.30 ± 0.19, n = 7) (Table 1) in male rats. Pre-treatment of yohimbine did not reverse the inhibition of gastric emptying (31.0 ± 5.0%, n = 6 versus the amphetamine group 21.9 ± 5.0%, n = 8) (Table 1) and intestinal transit (geometric center values: 1.68 ± 0.07, n = 6, versus the amphetamine group 1.82 ± 0.21, n = 8) (Table 1) by amphetamine.

Effects of Propranolol on the Inhibition of Gastric Emptying and Intestinal Transit by Amphetamine

Propranolol (1 mg/kg) had no effects on gastric emptying (69.4 ± 3.2%, n = 8, versus the saline group 69.4 ± 3.2%, n = 8) (Fig. 4A) and intestinal transit (geometric center values: 3.13 ± 0.25, n = 8, versus the saline group 3.23 ± 0.13, n = 8) (Fig. 4B) in male rats. Pre-treatment of propranolol significantly attenuated (48.4 ± 4.6%, n = 8) ($P < 0.05$) the inhibition of gastric emptying by amphetamine (19.4 ± 5.2%, n = 8) (Fig. 4A) but did not significantly attenuated (2.44 ± 0.18, n = 8) the inhibition of intestinal transit by amphetamine (1.98 ± 0.24, n = 6) (Fig. 4B).

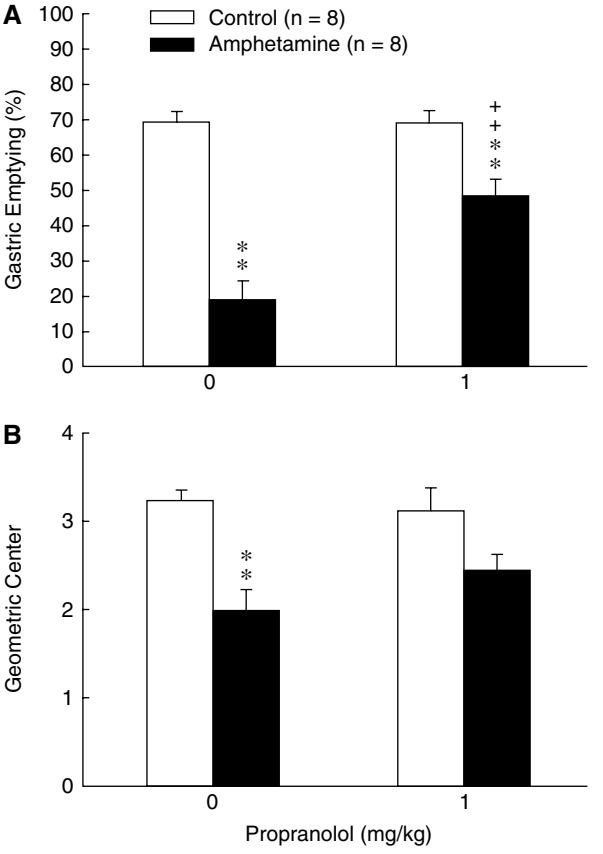

Fig. 4. Effects of propranolol on the inhibition of gastric emptying (A) and intestinal transit (B) by amphetamine in male rats. On the experiment day, animals received i.p. injection of normal saline (blank column, n = 8) or amphetamine (3 mg/kg) (solid column, n = 8) 15 min after i.p. injection of normal saline or propranolol (1 mg/kg). Thirty minutes later, all rats were given the test meal. Fifteen minutes later, rats were decapitated. See the legend to Fig. 1 for further details. Each column represents the means ± SEM. $^{**}P < 0.01$ compared with saline-injected rats. $^{++}P < 0.01$ compared with saline plus amphetamine group.

Discussion

Amphetamine, a CNS stimulant, is broadly used in drug addiction and treatment of obesity. It acts on the CNS by increasing levels of norepinephrine, serotonin and DA in the brain. It stimulates the colonic motility and GI function (11, 29) *via* the brain-gut connections (15). Previously, amphetamine was more popularly used to diminish appetite to control bodyweight. Whether suffering obesity or not, someone who takes amphetamine orally always promotes loss of bodyweight (3, 6, 14). Bodyweight loss is mediated by the inhibition of food intake (32). It was found that repeated treatment with amphetamine decreased food intake *via* DA action on D_1 and D_2 receptors (9). Wellman found that the dose of 3 mg/kg amphetamine inhibited food intake and had no side effects (33). Our study investigated that acute pretreatment of amphetamine at the effective doses (0.75 - 3 mg/kg) inhibited gastric emptying and intestinal transit in male rats. These results were correspondent to other similar researches on humans (13) and rodents (7). That amphetamine reduces appetite is mediated by the inhibition of gastric emptying (10). Moreover, amphetamine inhibits the gastric emptying and prolongs the ingestion time of nutrients in the stomach. The satiety messages from stomach to brain decreases the speed of food intake and inhibits appetite (32, 33). Therefore, the appetite inhibition of amphetamine is partly due to the inhibition of gastric emptying and intestinal transit.

An early study showed that the reduction of food intake and losing of appetite were partly due to action on the DA receptors and adrenoreceptors (5). It has been demonstrated that DA, as a neurotransmitter in the brain, is also present in the gastroduodenal mucosa and has been implicated in several functions in GI tract (23). D_1 and D_2 receptors proteins are present in the stomach through the distal colon but motility is abnormal when D_2 receptor is absent (19). Furthermore, amphetamine is a DA agonists which stimulates the release of DA. There is evidence that pretreatment of DA antagonist attenuates the effect of appetite losing (5). Our results showed that pretreatment of haloperidol completely reversed ($P < 0.01$) the inhibition of gastric emptying by amphetamine and prevented ($P < 0.01$) the inhibition of intestinal transit by amphetamine (Fig. 1). Pretreatment of SCH 23390 partially attenuated the inhibition of gastric emptying and intestinal transit by amphetamine. Pretreatment of eticlopride completely prevented the inhibition of gastric emptying and intestinal transit by amphetamine. Thus, it was summarized that amphetamine stimulated the DA release *via* the CNS, and that the effect of eticlopride on blocking D_2 receptor was more effective than the SCH 23390 in blocking D_1 receptors in inhibition of gastric emptying and intestinal transit by amphetamine.

Stimulation of α_2 adrenoreceptor inhibits the food intake (31). Motility and food intake are inhibited by α_2 adrenoreceptor agonists (22) which enhance gastrin release and inhibit gastrin-stimulated acid secretion in humans (30). Based on the effects of food intake and gastric acid secretion, sympathetic and parasympathetic nervous systems might be closely related to GI motility. Amphetamine has a sympathomimetic action (1, 21, 26), hence, the use of the adrenoreceptor antagonists can explore whether the inhibition of gastric emptying and intestinal transit by amphetamine are mediated through the sympathetic nervous system. Moreover, in 1974, scholars found that the down-regulation of amphetamine were blockade by α adrenoceptor or DA receptor blocking agents. However, which type of DA receptor and adrenoceptor was involved in the mechanism of amphetamine in GI function and emptying was unclear. Our data showed that pretreatment of α, α_1 and α_2 adrenoreceptor antagonists had no effects on the inhibition of gastric emptying and intestinal transit by amphetamine. Pretreatment of β adrenoreceptor antagonist partly attenuated the inhibition of gastric emptying but had no effects on the inhibition of intestinal transit by amphetamine. It has been known that the intestinal transit is affected by the quantity of foods (24). The β adrenoreceptor antagonist has no effect on the inhibition of intestinal transit by amphetamine. Although the sympathetic nerves control the inhibition of gastrointestinal motility, it was only the β adrenoreceptor that was involved in the mechanism, excluding the α, α_1 and α_2 adrenoreceptors.

Functional interactions between DA receptor subtypes may affect behavioral and biochemical responses (5). D_2 but not D_1 receptor densities are correlated with behavioral responses of animals to apomorphine (5). In the GI tract, physiological modulation of intestinal motility is mostly by enteric dopaminergic neurons and the D_2 receptor (19). These evidences consistent with our results. In conclusion, these results suggest that amphetamine-induced inhibition of gastric emptying and intestinal transit are mediated by an action on dopaminergic mechanism, and the D_2 receptors play a particularly important role.

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