

Original article

A simple method to investigate the inhibitory effects of drugs on gastric emptying in the mouse in vivo

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Abstract

Introduction: The aim was to develop a simple method to study modification of gastric motility in the mouse in vivo. **Methods:** Mice were fed a hydrated diet in which the fluid content of standard laboratory chow was increased by adding water. Gastric emptying was assessed at specified times following a 1-h treatment period with orally administered pharmacological agents. **Results:** We demonstrated consistent and progressive gastric emptying over a 4-h period, stomach content being decreased from 7.52 ± 0.90 at time zero to 2.80 ± 0.25 mg/g body weight after 4 h. Results demonstrated typical effects of inhibitory agents (atropine and morphine) and showed inhibitory effects of three potassium channel opening agents, pinacidil, cromakalim, and SDZ PCO400: the residue remaining in the stomach was increased by 3.66 ± 0.84 , 6.56 ± 1.35 , and 5.68 ± 1.33 mg/g body weight respectively 1 h after treatment with 10 mg/kg of these agents, compared to vehicle controls. **Discussion:** The inhibitory activity observed correlated well with previous studies on the effects of potassium channel opening agents on mouse gastrointestinal motility in vivo and in vitro. The present model may thus be of value in the pharmacological investigation of gastrointestinal motility owing to cost and convenience advantages, together with the possibility of its application to studies using transgenic animals. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Gastric emptying; Inhibition of gastric motility; Methods; Mouse; Potassium channel openers; Stomach

1. Introduction

The modification of mouse intestinal activity by various potassium channel openers has been extensively studied in our laboratories in vitro and in vivo (Yeung, McCurrie, & Wood, 1995a,b, 1996). However, the profile of activity of the potassium channel openers on the intact gastrointestinal tract remains incomplete owing to a lack of data on their actions on gastric motility. In the present experiments, a simple method for measuring gastric emptying in the mouse in vivo was developed for this purpose.

Several in vivo methods for assessing gastrointestinal transit have been reported previously. These include measurement of the passage of a radiolabelled solid meal in rats (Purdon & Bass, 1973) and human subjects

(Figuroa, Jordan, & Bassett, 1968; Bryk & Roska, 1969; Read, Al Janabi, Bates, & Barber, 1983) and breath hydrogen analysis which utilises a non-absorbable carbohydrate meal (Bond & Levitt, 1975; Brown, Rumsey, & Read, 1987). Most in vivo studies of the effects of pharmacological agents on gastric emptying have employed these techniques in the rat (Giri & Rice, 1970; Gelencser et al., 1973; Purdon & Bass, 1973; Franklin, 1977). However, the methods are complex and time-consuming.

Droppleman, Gregory, and Alphin (1980) reported a simpler method for studying the effects of drugs on gastric emptying which involves administration by gavage of a methylcellulose-based test meal to the rat. Our aim was therefore to adapt this method to mice, since a comparison of the effects of potassium channel openers on gastric emptying in vivo with the effects of these agents on both motility in vitro and intestinal transit in vivo in a single species has been hitherto lacking.

In preliminary experiments using this method in mice, substantial difficulty was experienced in feeding the test meal. The introduction of a semi-solid meal directly into the

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mouse stomach via the small gauge needle required was technically difficult and appeared to stress the animal. A simple new method for measuring gastric emptying, in the mouse, has therefore been developed and applied in the determination of the effects of smooth muscle relaxant agents on the transit of a meal from the stomach into the small intestine. The method involves the consumption of a hydrated form of standard laboratory chow by unrestrained, freely feeding mice.

Briefly, the method involves feeding mice a hydrated diet and assessing gastric emptying at specified times following a 1 h treatment period with orally-administered pharmacological agents: emptying is calculated by considering the weight of the full stomach, the empty stomach, and the animal. The model was characterized by examining the effects of potassium channel openers on mouse gastric emptying. Potassium channel openers have previously been reported to reduce gastrointestinal motility in a variety of species (human colon, Huizinga, 1986; canine colon, Huizinga, 1991; rat stomach, Lefebvre & Horacek, 1992; guinea-pig intestine, Zini, Ben-Ari, & Asford, 1991), but their effects in the mouse have yet to be characterised.

The advantages of the new method include minimal handling and stress on the animals and the ability to include a relatively large number of animals in each test group at reasonable cost. The method may prove useful in assessing the activity of other agents which possess inhibitory actions on gastrointestinal motility, and has the advantage of being conveniently adaptable to studies using transgenic animals.

2. Methods

2.1. Preparation of the test meal and the animals

Male Bantin and Kingman White mice weighing 30–50 g were obtained from the Bradford University Breeding Unit. All mice were initially housed in groups of ten in polypropylene cages with water and pelleted standard laboratory chow (CRM Rat & Mice Diet Services, Cheshire, UK) provided *ad libitum*. 24–36 h before commencing the experiment, groups of 10 mice were introduced to a hydrated form of standard laboratory chow (hydrated diet). This was prepared by placing 45 g of pellets in 100 ml water and storing in the refrigerator at 4°C for at least 16 h. Before dispensing the food to the animals, a further 2 ml water was added if necessary: this was to ensure that the food was saturated with water yet maintained a semi-solid state. Each group of animals received this quantity of chow at intervals of 8–12 h. The animals were maintained on the hydrated diet for 24–36 h prior to commencement of the experiments in order to allow them to adapt to the new food. Just before beginning the experiment each group of 10 mice was

transferred to a clean cage fitted with a grid floor. Water was provided *ad libitum* throughout the experimental period. The animals were left to adapt to the new cage for 1 h, after which time hydrated diet (prepared as described above) was placed in the centre of the cage. All animals were allowed equal access to it for 1 h (Free-Feeding Period). The hydrated diet was initially introduced because it was observed that in mice fed on standard dry chow, gastric emptying was very slow. The weight of residual food in the stomach of the animals following 24 h of food deprivation was still very substantial, even with a grid floor in place, and this made evaluation of gastric emptying difficult.

2.2. Experimental protocol

The weight of food residue present in the stomach of mice receiving a normal solid diet was assessed in animals subjected to three different conditions. The three groups of 10 mice were allocated as follows: (a) normal, non-food deprived; (b) food-deprived for 24 h in a standard, solid floor polypropylene cage; and (c) food-deprived for 24 h in a cage with a grid floor to prevent coprophagy.

2.3. Kinetic studies

The amount of residue in the stomach would be expected to be inversely proportional to the period of time following food consumption and to the rate of gastric emptying. Gastric emptying was compared in mice consuming either a normal or a hydrated diet. An identical method was used both in mice fed with normal dry pellets without food deprivation and in animals fed on a hydrated diet for 24–36 h.

2.4. Mice fed a normal diet

Groups of 10 mice receiving normal food pellets were transferred to clean polypropylene cages with grid floors for a 1-h habituation period prior to the commencement of the experiment. The mice remained in these cages with food removed but with a water supply maintained, for a time interval of 0, 1, 2, or 4 h. The animals were then sacrificed, laparotomised, and the stomach removed. The full stomach was weighed on an analytical balance. Each stomach was cut open and rinsed with water. Excess moisture was removed by gentle sponging with laboratory tissue and the empty stomach weighed. Three weights were required to assess gastric emptying: (a) weight of the full stomach; (b) weight of the empty stomach; and (c) body weight. The weight of food remaining in the stomach was expressed per gram body weight in order to take into consideration the variation in body size which in rats has been shown to influence the passage of a meal through the gastrointestinal tract (Purdon & Bass, 1973).

2.5. Mice fed a hydrated diet

After the 1-h free-feeding period, groups of mice were sacrificed at 0, 1, 2 or 4 h. Stomach and body weights were recorded as described above.

2.6. Pharmacological studies

The effects of the potassium channel openers, pinacidil (0.1–10 mg/kg), SDZ PCO400 (0.1–30 mg/kg) or cromakalim (0.1–10 mg/kg), or appropriate vehicle (10% alcohol maximum) were assessed on groups of mice receiving the hydrated diet. Animals were permitted 1 h of free feeding before the experiment. After removal of food, there followed a 1-h treatment period with a potassium channel opener (0.1 ml/10 g body weight) administered intragastrically using an oral 22 gauge dosing needle. The animals were then sacrificed. A selection of agents which have been shown to cause either an increase in gastric emptying, namely carbachol (Ruwart, Klepper, & Rush, 1978) and metoclopramide (Purdon & Bass, 1973), or a decrease in gastric emptying; atropine (Purdon & Bass, 1973) and morphine (Schulz, Wuster, & Herz, 1979) were investigated in the same way. Control experiments were also performed in which a volume of saline or a volume of vehicle (up to 10% alcohol) equal to the volume of drug used was placed in the stomach. The influence of these agents on the amount of residue remaining in the stomach was thus assessed 2 h after feeding.

2.7. Drugs and chemicals

Pinacidil ((±)-N-cyano-4-pyridyl-N-1,2,2-trimethyl-propyl-guanidine mono-hydrate) and SDZ PCO400 ((3S,4R)-3, 4-Dihydro-3hydroxy-2,2-dimethyl-4-[(3-oxo-1-cyclopenten-1-yl)oxy]-2h-1-benzopyran-6-carbonitrile) were generous gifts from Leo Pharmaceuticals (Princes Risborough, Buckinghamshire, UK) and Sandoz Pharmaceuticals respectively. Cromakalim ((±)-6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2H-benzo[bepyrans-3-ol]) and all other agents were obtained from Sigma-Aldrich Co. Ltd. (Poole, Dorset, UK).

2.8. Expression of results and statistics

Results were expressed as stomach content in milligrams per gram body weight (mg/g BW), calculated as follows:

$$\frac{(\text{full stomach weight} - \text{empty stomach weight})}{\text{body weight}}$$

The results are expressed as mean \pm SEM and statistical significance of differences was calculated using the Student's unpaired *t*-test. When three or more groups were to be compared, analysis of variance (ANOVA) was used, followed by Dunnett's *t*-test.

3. Results

3.1. Development of the method to assess gastric emptying

In preliminary experiments, attempts were made to assess the emptying of mouse stomach using the method developed for rat by Droppleman et al., (1980) using a methylcellulose-based test meal administered intragastrically to food-deprived animals. Two major problems were encountered in attempting to apply the method in mice. First there were difficulties in attempting to administer the semi-solid paste through the requisite small gauge needle. Second, there was very slow emptying of normally ingested dry food over the preliminary 24-h food deprivation period so that assessment of any delay in emptying of the methylcellulose meal was especially difficult. Coprophagy may be partly responsible for the quantity of food remaining in the stomach after 24 h of food deprivation since, in a series of experiments in which mice were placed in a cage with a grid floor, the residue in the stomach after 24-h food deprivation was markedly reduced. The stomach residue decreased from 8.2 ± 1.8 mg/g BW in the mice held in cages with a solid floor to 3.4 ± 0.5 mg/g BW when a grid floor was used ($P < .001$, $n = 10$).

In an attempt to overcome these difficulties, standard laboratory chow was mixed with water in order to increase the speed of gastric emptying and facilitate the transit of the test meal into the small intestine.

3.2. Kinetic studies

The extent of gastric emptying in mice fed the hydrated diet was found to be time-dependent. The weight of stomach contents was reduced from 7.52 ± 0.90 mg/g BW at time zero to 2.80 ± 0.25 mg/g BW at 4 h (Fig. 1). In comparison,

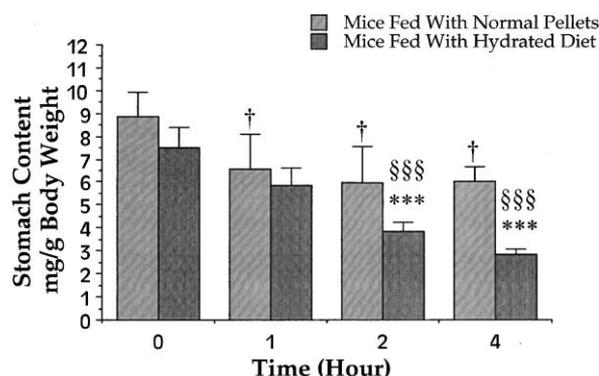


Fig. 1. The extent of gastric emptying was assessed in two groups of mice which were allowed access to either normal food pellets or the hydrated diet for 24–36 h prior to commencement of the experiments. Groups of mice were sacrificed at 0, 1, 2 or 4 h. Each column represents the mean of 9 to 10 animals and vertical bars represent the SEM. The results were analysed using ANOVA followed by Dunnett's *t*-test. *** $P < .001$, mice fed with hydrated diet at time = 2 and 4 h vs. time = 0 h; † $P < .05$, mice fed with normal diet at time = 1, 2, and 4 h vs. time = 0 h; §§§ $P < .001$, mice fed with normal diet vs. mice fed with hydrated diet at the corresponding time intervals.

mice fed on normal dry chow showed a much slower pattern of gastric emptying which appeared not to be time dependent over the 4-h observation period (Fig. 1).

3.3. The effects of carbachol, atropine, morphine, and metoclopramide on mouse gastric emptying

Oral administration of a volume (approximately 0.3–0.4 ml) of saline or vehicle caused an increase in the rate of gastric emptying. In the saline control 2.87 ± 0.65 mg/g BW ($n=9$) stomach content remained and in vehicle controls 2.88 ± 0.32 mg/g BW ($n=10$) was present 1 h after saline or vehicle treatment. These values were significantly lower than the residual content observed 1 h after cessation of feeding in the untreated mice (5.88 ± 0.74 mg/g BW, $n=10$). Since the volume in which drugs were administered caused an increase in the rate of emptying all results were compared to the stomach residue remaining after saline or vehicle control experiments as appropriate.

Atropine (10 mg/kg) and morphine (10 mg/kg) both caused significant reduction in gastric emptying compared to the saline control (Fig. 2). Carbachol (10 mg/kg) and metoclopramide (10 mg/kg), which are known to stimulate gastric emptying in the rat, did not cause significant increase in the emptying rate in the mouse (Fig. 2). However, the actual volume in which drugs were administered significantly increased the rate of emptying, which may have reached maximal, so masking any stimulatory effects of carbachol and metoclopramide.

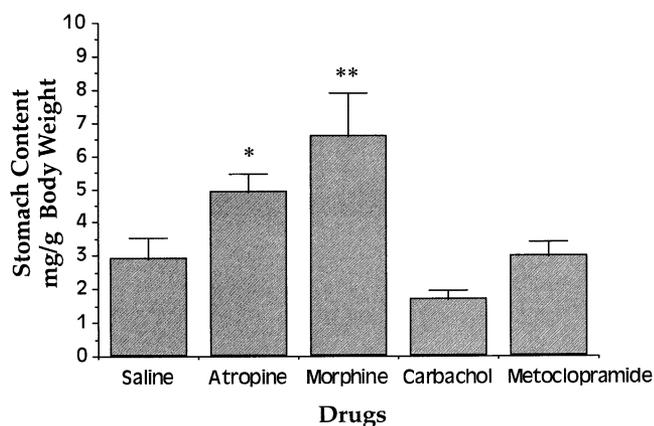


Fig. 2. The effects of atropine (10 mg/kg), carbachol (10 mg/kg), metoclopramide (10 mg/kg), morphine (10 mg/kg) or saline administered intragastrically (0.3 ml total volume) were investigated in mice fed on the hydrated diet, with 1 h drug treatment time before termination of the experiments. The amount of stomach content remaining using this diet alone was 5.88 ± 0.74 mg/g BW as shown in Fig. 1. Each bar represents the mean of nine experiments except in the case of atropine where $n=19$ and the vertical bars represent SEM. The results were analysed using Student's unpaired *t*-test. * $P < .05$, ** $P < .001$ drugs vs. saline.

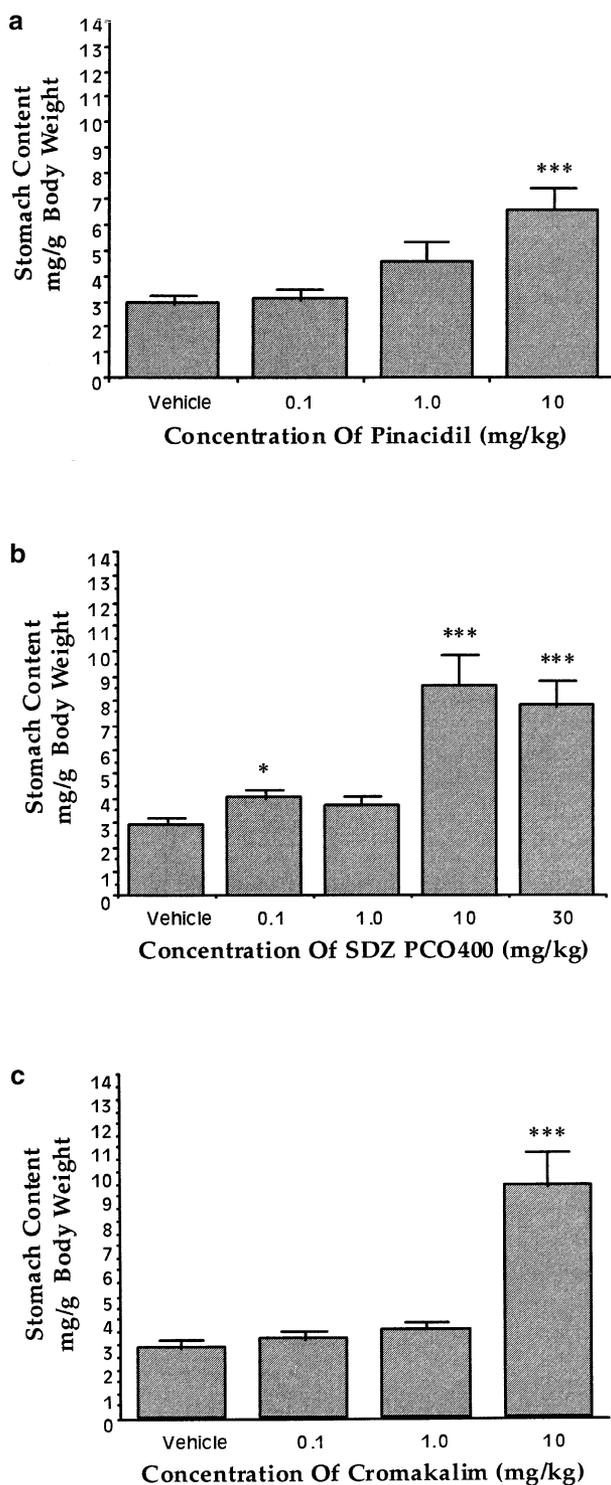


Fig. 3. The effects of (a) pinacidil, (b) SDZ PCO400, and (c) cromakalim on gastric emptying were investigated in mice which had been fed with the hydrated diet. The agents were administered intragastrically (0.3 ml total volume) with 1 h treatment period after the initial 1-h free-feeding period. Each bar represents the mean of stomach content (mg/g BW) in 8–10 experiments and the vertical bars represent SEM. The results were analysed using Student's unpaired *t*-test. * $P < .01$, *** $P < .001$ vs. vehicle (10% alcohol).

3.4. Effects of potassium channel openers on gastric emptying in mice

Pinacidil (0.1–10 mg/kg), SDZ PCO400 (0.1–30 mg/kg), and cromakalim (0.1–10 mg/kg) were administered orally. The highest doses of each agent caused inhibition of gastric emptying equal to or greater than that caused by morphine (10 mg/kg) as shown in Fig. 2. Pinacidil and cromakalim were ineffective at low doses (0.1–1.0 mg/kg) (Fig. 3), but SDZ PCO400 appeared to be a somewhat more effective inhibitor, showing a small but significant reduction in gastric emptying at 0.1 mg/kg. These inhibitory effects on gastric emptying were apparent within 1 h of drug administration. Higher doses of the above agents were not administered since the drugs could not be completely dissolved in the small volume of vehicle required.

4. Discussion

The present study characterised a simple, direct method of assessing gastric emptying in the mouse with minimal handling and stress for the animals. Gastric emptying was delayed by both atropine and morphine (10 mg/kg) and by pinacidil, cromakalim, and SDZ PCO400 at the highest doses used. However, stimulants of gastric motility, carbachol, and metoclopramide (10 mg/kg), did not significantly increase the rate of emptying in these studies.

The new method demonstrated consistent and progressive emptying of mouse stomach over a 4-h period following a free-feeding period. A drug treatment interval of 1 h following the initial free-feeding period was chosen since at this time the residual food in the stomach, determined by the kinetic study, was sufficient to allow either an increase or decrease in the emptying rate to be measured. However, control experiments using saline or vehicle showed that the small volume in which the drugs were to be delivered by gavage (0.3–0.4 ml) caused a significant increase in gastric emptying, measured 1 h after ingestion. The increase in emptying rate caused by this volume was marked: the stomach content remaining 1 h after oral dosing with saline or vehicle being similar to that measured 4 h after feeding the hydrated diet alone. It has been previously reported that the passage of a meal through the gastrointestinal tract is influenced both by the volume of the meal and the body weight of the test animal (Purdon & Bass, 1973). Furthermore, the administration of pharmacological agents by gavage and/or the stress caused by handling the animals might also be expected to increase the rate of gastric emptying (Cann et al., 1983; Stanghellini, Malagelada, Zinsmeister, Go, & Kao, 1983). Therefore, in those experiments, which involved oral administration of pharmacological agents, all results were compared with appropriate saline or vehicle controls.

Pinacidil, cromakalim, and SDZ PCO400 all at the highest dose used (10 mg/kg) caused similar inhibition of gastric

emptying. The effects were qualitatively similar to data obtained from a previous *in vivo* study on intestinal transit using the charcoal meal test (Yeung et al., 1996). The lack of inhibition observed with the lower doses of these agents (0.1 and 1.0 mg/kg) may reflect an inability to overcome stimulation of emptying caused by the factors mentioned above, although in the case of pinacidil and cromakalim, this is consistent with clinical data: in clinical trials with cromakalim (1.5 mg daily) and pinacidil (up to 75 mg daily) for treatment of hypertension, no major gastrointestinal side effects were reported (Van den Burg, Woodard, Steward-Long, Tasker, Pilgrim, Dews, & Fairhurst, 1987; Goldberg, 1988). Other agents known to reduce contractile activity in the gut, atropine (10 mg/kg) and morphine (10 mg/kg), significantly reduced gastric emptying in the present study. Although it has been reported that the stimulatory agents carbachol (Ruwart et al., 1978) and metoclopramide (Purdon & Bass, 1973) increase gastric emptying in the rat, these agents showed no effects on mouse gastric emptying in the present study. This may be related to the marked stimulation of emptying caused by the ingestion volume itself, which may have minimized scope for detecting further stimulation, or to other factors, such as the dose of agent used. These factors require further investigation. Presently, however, this means that the method can be recommended only for detecting the actions of inhibitory agents.

As described earlier, inhibitory effects of the potassium channel openers, pinacidil and cromakalim, on gastric motility could be demonstrated only at the highest doses used (10 mg/kg). The lack of a dose-related response to these agents over the same range was also observed in the charcoal meal test for measurement of gastrointestinal transit (Yeung et al., 1996). This suggests that the inhibitory effects of these agents on gastrointestinal transit, as observed in the charcoal meal test, may to some extent reflect their actions on gastric emptying. In fact it has been demonstrated that, under normal conditions, the rates of gastric emptying and intestinal transit are identical in the proximal 60% of the small intestine (Grevsten, Johanssen, Linquist, & Nylander, 1969)

Pharmacokinetic factors are likely to contribute to drug effects on motility. In man, pinacidil is rapidly absorbed following oral administration, bioavailability is 57% and peak plasma concentration occurs within 1 h (Andersson, 1992). Cromakalim has a longer half-life than pinacidil but is more slowly absorbed, attaining peak plasma concentration after 1–4 h (Davies et al., 1988). No pharmacokinetic data appears to be available for SDZ PCO400. However, since the effect of all three agents on gastric emptying was similar in the present study, it would appear likely that pharmacokinetic differences between the drugs exert less influence on gastric emptying than the many other factors likely to contribute in this model, discussed above.

The present study has shown that the rate of gastric emptying in the mouse depends both on the texture and volume of 'standard meal'. In preliminary experiments (not shown) when the standard meal was dry, emptying was very

slow and the stomach contained a large amount of food after a 24-h food deprivation period. Increasing the water content of the standard meal increased the rate of gastric emptying, which was consistent over the 4-h observation period.

Orally-administered drug vehicle caused a marked increase in gastric emptying rate. This indicates that vehicle volume must be standardized both within and between studies. In addition, the volume effect has the potential to reduce scope for detecting the effects of agents that genuinely stimulate gastric motility. However, the method was effective in demonstrating the ability of a number of different pharmacological agents to slow gastric emptying in the mouse. The protocol described has the advantage of not requiring a food deprivation period or a test meal delivered by gavage, so reducing the stress on the animals. Stress has been shown to affect human gastric emptying (Stanghellini et al., 1983) and mouth to caecum transit time (Cann et al., 1983). As in other species, a large number of variables may affect results obtained in vivo and relatively large test groups (≥ 8) are required to show significant changes in gastrointestinal motility in the mouse. However, the method described is simple, economical, and requires no prior administration of a stimulatory agent or special equipment. The present study successfully demonstrated inhibitory effects of three different potassium channel openers on gastric emptying, in agreement with previous reports (Huizinga, 1986; Huizinga, 1991; Zini et al., 1991; Lefebvre & Horacek, 1992). These results also accord with our previous studies in the mouse which showed relaxant effects of potassium channel openers on the intestine in vitro (Yeung et al., 1995a,b) and inhibitory effects on gastrointestinal transit in vivo (Yeung et al., 1996).

In summary, although the method developed by Droppleman et al. (1980) for measuring gastric emptying using a methylcellulose test meal is unsuitable for use in mice, the method described here was found to be practical, capable of detecting inhibitory drug actions, economical, and simple.

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