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Contrasting effects of tetraethylammonium and 4-aminopyridine on the gastrointestinal function of mice

De-Li Dong*, Qing-Hui Wang, Wei Chen, Jiao-Jie Fan, Jing-Wei Mu, Jia Ke, Bao-Feng Yang*

Department of Pharmacology, Harbin Medical University; Bio-pharmaceutical Key Laboratory, Baojian Road 157, Harbin 150086, Heilongjiang Province, China

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Abstract

Many different K^+ channels have been identified in the gastrointestinal tract, and the two classical K^+ channel blockers, tetraethylammonium and 4-aminopyridine, show different sensitivity for these channels. The aim of the present study was to compare the effects of tetraethylammonium and 4-aminopyridine on the gastrointestinal function of mice. 4-Aminopyridine (5 mg/kg, p.o.) inhibited, but tetraethylammonium (40 mg/kg, p.o.) enhanced, the intestinal propulsion of a charcoal suspension in conscious mice. Studies in vitro showed that perfusion of 5 mM 4-aminopyridine increased the maximal contractile force and minimal relaxation force, and decreased the amplitude and frequency of the peristaltic contraction of the isolated duodenum. However, perfusion of 5 mM tetraethylammonium increased the maximal contractile force, the minimal relaxation force and the amplitude of the contraction. The effects of tetraethylammonium and 4-aminopyridine on the duodenal contraction could be abolished completely by application of 5 μM verapamil. Our results in vivo and in vitro showed that tetraethylammonium and 4-aminopyridine had contrasting effects on the gastrointestinal function of mice.

Keywords: Tetraethylammonium; 4-aminopyridine; Gastrointestinal motility; Gastric acid secretion

1. Introduction

Many different K^+ channels, such as large conductance (Carl and Sanders, 1989) and small conductance (Koh et al., 1997; Vogalis and Goyal, 1997) Ca^{2+}-activated K^+ channels, inwardly rectifying (Benham et al., 1987), and ATP-sensitive (Koh et al., 1998), voltage-dependent Ca^{2+}-insensitive delayed rectifier K^+ currents (Jaggar et al., 1998; Thornbury et al., 1992) have been identified in several regions of the gastrointestinal tract. A-type K^+ currents have also been identified in several gastrointestinal smooth muscles, including guinea-pig colon (Vogalis et al., 1993), opossum esophagus (Akbarali et al., 1995), mouse colon (Koh et al., 1999), and mouse antrum (Amberg et al., 2002). In gastrointestinal smooth muscles, electrophysiological recordings provide evidence for components of I_{Kr}- and I_{Ks}-like channels (Akbarali et al., 1999; Benham and Bolton, 1983), Ohya et al. reported that ERG1 (ether-a-go-go-related K^+ channel)/KCNE2 (potassium channels encoded gene) transcripts were expressed in rat stomach fundus and antrum smooth muscle cells, and that KCNQ1/KCNE1 (potassium channels encoded gene) transcripts were expressed in the antrum but not in the fundus. ERG1 proteins were substantially expressed in both regions, whereas KCNE1 proteins were faintly expressed in the antrum but not in the fundus (Ohya et al., 2002). Epperson et al. reported the differential expression of eighteen different K_V channel genes in canine gastrointestinal smooth muscle cells (Epperson et al., 1999). These data suggest that the wide array of electrical activity found in different regions of the gastrointestinal tract may be due in part to the differential expression of K^+ channels.

* Corresponding authors. Tel.: +86 451 86671354; fax: +86 451 86675769.
E-mail address: delidong2004@yahoo.com.cn (D.-L. Dong).

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Tetraethylammonium and 4-aminopyridine are classical K^+ channel blockers. In isolated guinea-pig colonic (Vogalis et al., 1993) and opossum esophageal myocytes (Akbarali et al., 1995), inhibition of the A-type current with 4-aminopyridine shifted the resting membrane potential to more positive potentials and increased the velocity of the action potential upstroke. In mouse colon, application of 4-aminopyridine to intact preparations abolished the quiescent periods between slow waves and induced a slight depolarization (Koh et al., 1999). Tetraethylammonium, at low concentrations, blocks the maxi-K^+ channel (main Ca^{2+}-activated K^+ channel) relatively selectively. In canine and guinea pig gastric muscle cells, the sensitivity of the transient K^+ current to tetraethylammonium was lower than that of the classical maxi-K^+ channel (Noack et al., 1992). Snetkov et al. reported that, even in the same tissue (human bronchial smooth muscle cells), individual cells possessed pharmacologically different K^+ channels: some cells had 4-aminopyridine-sensitive (Ca^{2+}-independent) delayed rectifying K^+ channels, whereas others had tetraethylammonium-sensitive (Ca^{2+}-dependent) delayed rectifying K^+ channels (Snetkov et al., 1995). In rabbit ileal muscle, the delayed rectifying K^+ current was inhibited by 1 mM tetraethylammonium but not by 1 mM 4-aminopyridine, whereas the same current in the rabbit pulmonary artery was inhibited by 1 mM 4-aminopyridine but not by 10 mM tetraethylammonium (Ohya et al., 1986). The electrical properties of the stomach, small bowel, and colon differed in resting membrane potential, frequency, and waveform due to the qualitative and/or quantitative expression of ion channels expressed in each region and even within a region of the gastrointestinal tract (Sanders, 1992). Accordingly, tetraethylammonium and 4-aminopyridine may have different effects on gastrointestinal function due to their different sensitivity for subtypes of K^+ channels expressed in the gastrointestinal tract, but no systematic comparison has been reported until now. The aim of the present study was to illustrate the effects of tetraethylammonium and 4-aminopyridine on gastrointestinal function of murine in vivo and in vitro.

2. Materials and methods

2.1. Animals

Kunming mice and Wistar rats, weighing 20–22 g and 220–250 g respectively, were used. The mice and rats were housed 10 per cage under standard animal room conditions (temperature 21±1 °C; humidity 55–60%) with food and water continuously available for 1 week before the experiment. The animals were starved for 24 h before the experiment but had free access to water. All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, PR China.

2.2. Gastrointestinal transit

The effects of 4-aminopyridine (5 mg/kg, p.o.), tetraethylammonium (40 mg/kg, p.o.), atropine (1 mg/kg, p.o.), or vehicle (normal saline, 10 ml/kg, p.o.) on gastrointestinal transit were studied in conscious mice as described by Santos and Rao (1999). An aqueous charcoal suspension (10 ml/kg of a 5% activated charcoal suspension in 10% gum arabic) was given orally to each mouse, 20 min after saline or drug administration. The animals were killed by cervical dislocation under ethylether anesthesia 20 min after receiving the charcoal, and the stomach and duodenum were carefully removed without stretching and extended on a clean glass surface. The total length of the intestine from pylorus to rectum and the distance traveled by the charcoal were measured. Gastrointestinal transit is expressed as the distance traveled by the charcoal as a percentage of the total length from pylorus to rectum. Doses of drugs were selected based on our pilot experiments.

2.3. Preparation of gastrointestinal samples and contractive activity recording

Wistar rats (220–250 g) were killed by stunning and cervical dislocation. The abdomen was opened, and the stomach and duodenum were removed. The fundus was dissected and washed in fresh Krebs solution. Following this, longitudinal strips (1.5 cm long) were carefully prepared and mounted vertically in an organ bath (15-ml capacity) containing Krebs solution bubbled with air (37 °C, pH7.4). The duodenum (1.5 cm long) was prepared in the same way. The fundus muscle strips were mounted under a resting tension of 1 g before the experiment. The duodenum was mounted under an initial resting tension of 2 g and left to equilibrate for 1 h, following which a further 2 g was applied before the experimental protocol was started. Tension changes were recorded using isometric force transducers (ModelYH-4; Institute of Space Medical-Engineering, China) connected to a multichannel acquisition and analysis system (Model BL-420E, Taimeng Technology Instrument, Chengdu, China).

2.4. Measurement of gastric acid secretion

Wistar rats (220–250 g) were anesthetized with pentobarbital. Each experiment was started at least 1 h after implantation of the cannula. Gastric acid secretion was determined with gastric perfusion methods as previously reported (Minowa et al., 2004). The trachea was exposed, and cannulated, and the esophagus was ligated at the cardia. After laparotomy, the pylorus was ligated and a dual cannula was inserted into the gastric lumen from the gastric antrum. The stomach lumen was continuously perfused with saline (adjusted to pH 7.0, at 37 °C) at the rate of 0.2 ml/min through the inlet tube of the dual cannula connected to the perfusion pump. The stomach was maintained at a pressure

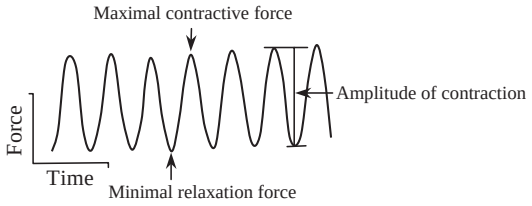

Fig. 1. Representative model of gastrointestinal contractions. Maximum contractile force, minimum relaxation force, amplitude of contraction, and frequency of contraction were determined as parameters of the contractile pattern of isolated tissues.

of 5 cm H_2O. After the determination of basal acid secretion for 2 h, tetraethylammonium (20 mg/kg, s.c.) and 4-aminopyridine (2.5 mg/kg, s.c.) were administered. The perfusate flowing from the outlet tube was collected for 2 h and was titrated to pH 8.0 with 0.01 M NaOH. The total acid output for 2 h was calculated for statistics.

2.5. Drugs and solution

Tetraethylammonium, 4-aminopyridine, verapamil, and atropine were obtained from Sigma (USA). The bathing solution was Krebs solution of the following composition (mM): NaCl 118.5, KCl 4.8, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.2, $NaHCO_3$ 25, Glucose 11. The solution was aerated with air, and the pH of the solution was maintained at 7.3–7.4.

2.6. Data analysis

Longitudinal strips of isolated fundus and duodenum contracted (as shown in Fig. 1). In order to describe the changes in the contractile pattern of the isolated tissues in detail after drug application, four parameters (as marked in Fig. 1) were determined: maximum contractile force, minimum relaxation force, amplitude of contraction, and frequency of contraction. For each sample, the mean of 20 continuously recorded values in the course of drug application and washout were normalized to that of a normal recording and the normalized data were used for statistical analysis.

Data are expressed as the means±S.E.M. Statistical analysis was performed using Student's *t*-test and values were taken to be significantly different when $P<0.05$.

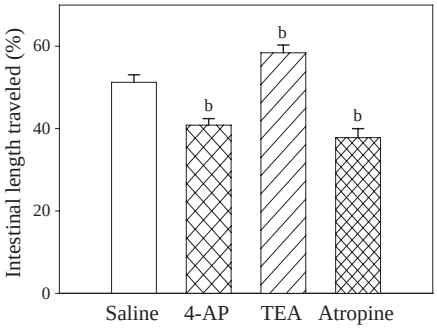

Fig. 2. Effects of 4-aminopyridine, tetraethylammonium, and atropine on intestinal transit in mice as assessed by charcoal propulsion. Animal numbers in the saline, 4-aminopyridine, tetraethylammonium, and atropine groups were 20, 22, 20, and 25, respectively. Results are presented as means±S.E.M.; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; $^{b}P<0.01$ vs saline.

3. Results

3.1. Effects of 4-aminopyridine and tetraethylammonium on gastrointestinal transit of mice

As shown in Fig. 2, the intestinal transit of the 4-aminopyridine group (40.9%±1.6%, n=22) was signifi-

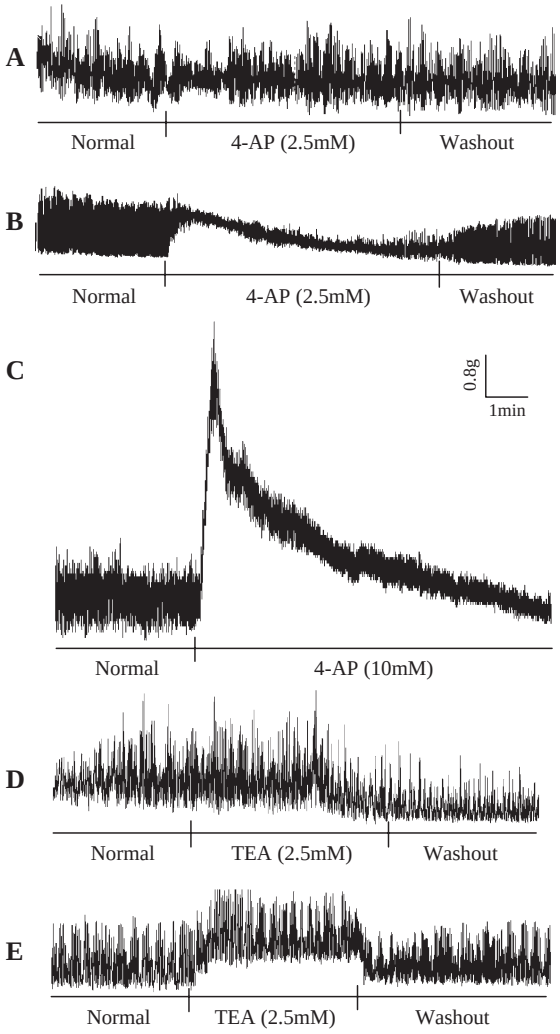

Fig. 3. Typical action of different concentrations of 4-aminopyridine and tetraethylammonium on rat duodenum contraction in vitro. A. Negative effect of 2.5 mM 4-aminopyridine on duodenum contraction in 10 of 25 samples. B. Positive effect of 2.5 mM 4-aminopyridine on duodenum contraction in 15 of 25 samples. C. Positive effect of 10 mM 4-aminopyridine on duodenum contraction in 7 samples. D. Negative effect of 2.5 mM tetraethylammonium on duodenum contraction in 6 of 24 samples. E. Positive effect of 2.5 mM tetraethylammonium on duodenum contraction in 18 of 24 samples. 4-AP, 4-aminopyridine; TEA, tetraethylammonium.

cantly less than that of the saline group (51.2%±1.8%, n=20, P<0.01). However, compared with the saline group, tetraethylammonium significantly enhanced the propulsion of charcoal, which traveled 58.4%±1.9% of the length of the intestine (n=20, P<0.01). Atropine was set as the positive control and the percentage of intestinal length traveled in the atropine group was 37.8±2.2 (n=15, P<0.01 vs saline), showing inhibitory effects on gastrointestinal transit.

3.2. Effects of 4-aminopyridine and tetraethylammonium on gastric motility of rats in vitro

Factors influencing gastrointestinal transit include gastric motility and intestinal motility, so the effects of tetraethylammonium and 4-aminopyridine on gastric motility of rats were studied first. Perfusion of 5 mM 4-aminopyridine had no significant effect on the maximal contractile force, amplitude and frequency of the contraction of longitudinal strips of isolated fundus, but the normalized minimal relaxation force of contractions was increased to 1.555±0.189 (P<0.05). Perfusion of 3 mM 4-aminopyridine had no effect on any of the above parameters.

Perfusion of tetraethylammonium at 1 mM, 3 mM, 5 mM, and 10 mM had no significant effect on the contraction of isolated fundus longitudinal strips. An increase in contraction frequency (normalized data was 1.495±0.132, P<0.05 vs normal) was observed up to 20 mM tetraethylammonium.

3.3. Effects of 4-aminopyridine and tetraethylammonium on intestinal motility of rats in vitro

Data for the concentration–response relationship showed that the effects of 4-aminopyridine and tetraethylammonium on the intestinal motility of rats were dose dependent. As shown in Fig. 3, 2.5 mM 4-aminopyridine showed negative actions in 40% (Fig. 3A) and positive action in 60% (Fig.

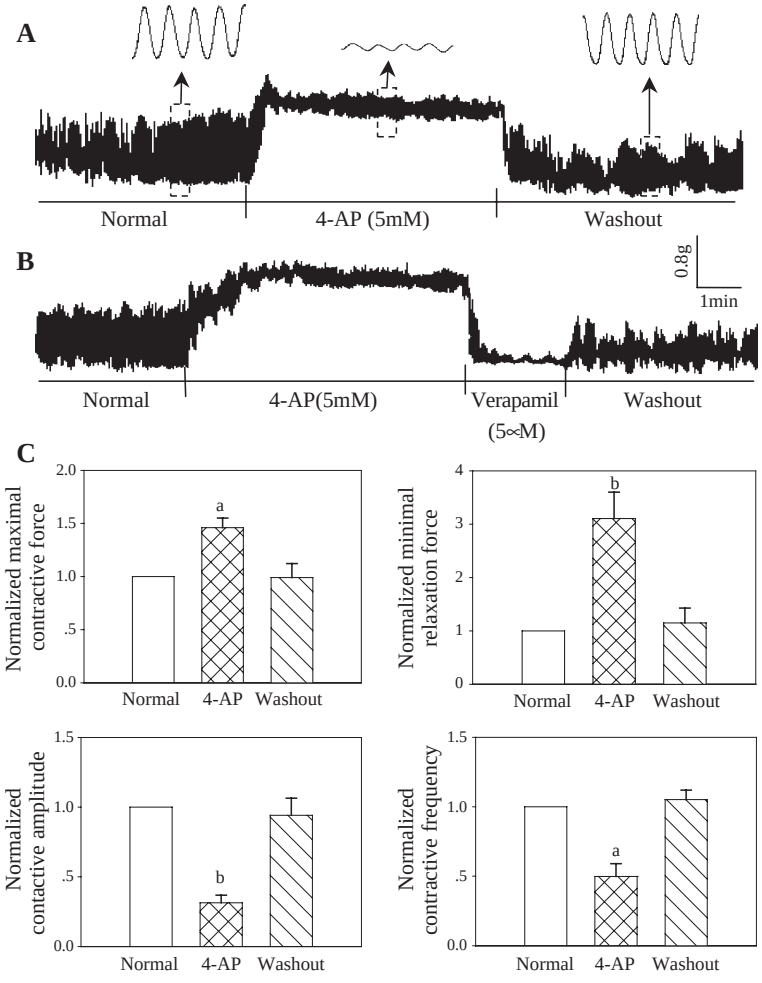

Fig. 4. Effects of 4-aminopyridine on rat duodenum contraction in vitro. A. Representative recording of rat duodenum contraction after application of 4-aminopyridine and washout. The boxed sections of the original recording are magnified, as indicated by the arrow. B. Representative recording of rat duodenum contraction after application of 4-aminopyridine (5 mM), verapamil (5μM), and washout. C. Normalized data of maximal contractile force, minimal relaxation force, amplitude, and frequency of the contraction after application of 4-aminopyridine (5 mM) and washout. Scaling in A and B is the same; 4-AP, 4-aminopyridine; n=11; [a]P<0.05, [b]P<0.01 vs normal.

3B) of 25 samples. When the concentration of 4-aminopyridine was increased to 10 mM, the force of contraction was enhanced dramatically in all 7 preparations (Fig. 3C). 4-Aminopyridine (5 mM) had a stable positive action in all 11 preparations (Fig. 4A). Tetraethylammonium (2.5 mM) had a negative action in 25% (Fig. 3D) and positive action in 75% (Fig. 3E) of 24 samples. Tetraethylammonium (5 mM) had stable positive effects in all 11 samples (as shown in Fig. 5A). Therefore, 5 mM 4-aminopyridine and tetraethylammonium was used to evaluate the action on intestinal motility.

Fig. 4A shows data for representative recordings of rat duodenum contraction after application of 4-aminopyridine and washout. 4-Aminopyridine (5 mM) significantly changed the contractile pattern of the duodenum and the changes were restored by washout of the drug. Further tests showed that the changes induced by 5 mM 4-aminopyridine were inhibited by application of 5 μM verapamil (Fig. 4B), indicating that L-type calcium channels were involved in the action of 4-aminopyridine on the duodenum contraction. The averaged data summarized in Fig. 4C show that perfusion of 5 mM 4-aminopyridine increased the maximal contractile force (1.460 ± 0.090, $n=11$, $P<0.05$) and the minimal relaxation force (3.107 ± 0.492, $n=11$, $P<0.01$), and decreased the amplitude (0.314 ± 0.055, $n=11$, $P<0.01$) and frequency (0.498 ± 0.092, $n=11$, $P<0.05$) of the contraction. All changes were restored by washout.

Fig. 5A shows data for representative recordings of rat duodenum contraction after application of 5 mM tetraethylammonium and washout. Tetraethylammonium (5 mM) significantly changed the contractile pattern of duodenum and the changes were restored by washout of

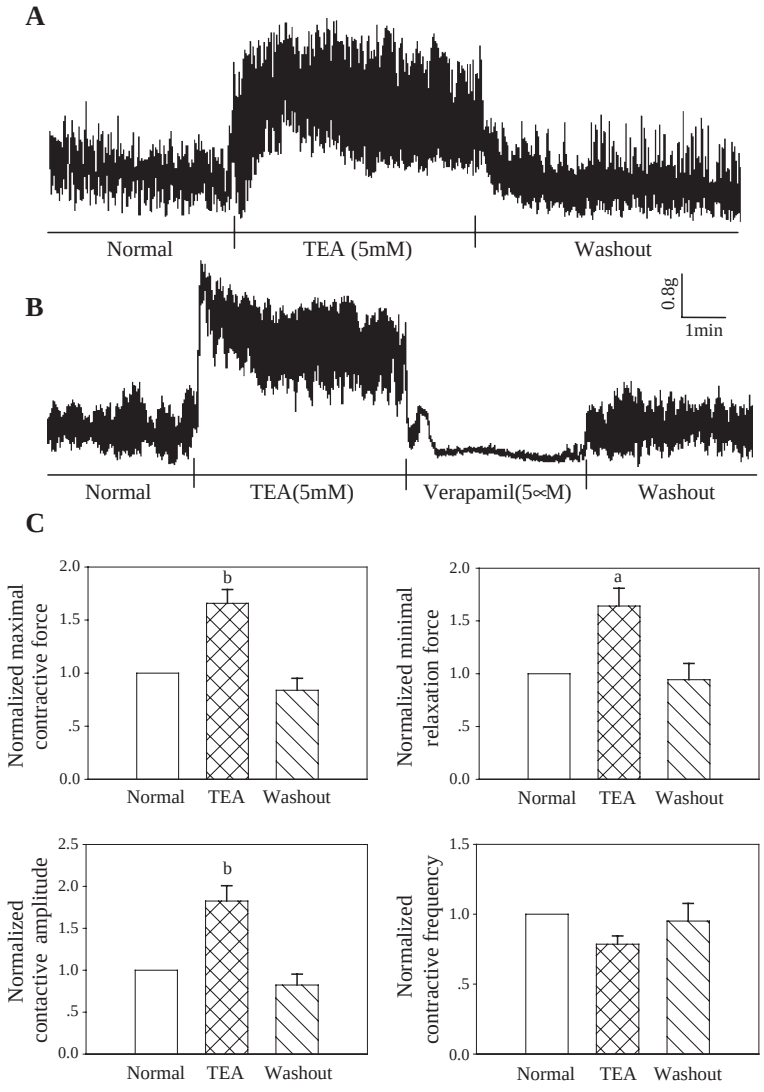

Fig. 5. Effects of tetraethylammonium on rat duodenum contraction in vitro. A. Representative recording of rat duodenum contraction after application of tetraethylammonium (5 mM) and washout. B. Representative recording of rat duodenum contraction after application of tetraethylammonium (5 mM), verapamil (5 μM), and washout. C. The normalized data for maximal contractile force, minimal relaxation force, amplitude, and frequency of the contraction after application of tetraethylammonium and washout. Scaling in A and B is the same; TEA, tetraethylammonium; $n=11$; $^{a}P<0.05$, $^{b}P<0.01$ vs normal.

the drug. Further tests showed that the changes induced by 5 mM tetraethylammonium were inhibited by application of 5 μM verapamil (Fig. 5B), indicating that L-type calcium channels were also involved in the action of 5 mM tetraethylammonium on duodenum contraction. The averaged data summarized in Fig. 5C show that perfusion of 5 mM tetraethylammonium increased the maximal contractile force (1.658±0.130, n=11, P<0.01), the minimal relaxation force (1.641±0.169, n=11, P<0.05) and the amplitude (1.825±0.182, n=11, P<0.01) of the contraction.

3.4. Effects of 4-aminopyridine and tetraethylammonium on gastric acid secretion of rats

Gastric acid secretion of rats was evaluated by the ratio of total acid output for 2 h after drug administration to basal acid output for 2 h. 4-Aminopyridine (2.5 mg/kg, s.c.) significantly increased gastric acid secretion: the normalized gastric acid output was increased to 2.179±0.351 (n=6, P<0.05, vs basal value). No effects were shown by tetraethylammonium treatment (20 mg/kg, s.c., n=7).

4. Discussion

The K^+ current contributes to the resting membrane potential, the duration of rhythmic activity, and the response of smooth muscle to neurotransmitters. A number of different K^+ channels have been identified in the gastrointestinal tract. Several authors reported that the classic K^+ channels blockers, tetraethylammonium and 4-aminopyridine, increased gastrointestinal contraction (Boev et al., 1985; Cheng et al., 1989; Velasco et al., 1997; Yuan et al., 1998), but they did not analyze the contractile pattern induced by these K^+ channel blockers. The present study found that tetraethylammonium and 4-aminopyridine had contrasting effects on the gastrointestinal motility of mice in vivo and in vitro. Our initial results demonstrating that tetraethylammonium enhanced, but 4-aminopyridine decreased, the gastrointestinal transit of mice in vivo focused our attention on the studies in vitro. Further investigation showed that 4-aminopyridine decreased the amplitude and frequency of gastrointestinal peristaltic contraction, and the spastic contraction induced by 4-aminopyridine was abolished by application of verapamil. Tetraethylammonium significantly increased the amplitude of intestinal peristaltic contraction, which might contribute to the enhancement of intestinal propulsion.

The hypothesis of the present study is that tetraethylammonium and 4-aminopyridine may have different effects on gastrointestinal function because of their different sensitivity for subtypes of K^+ channels expressed in the gastrointestinal tract. We showed experimentally that tetraethylammonium and 4-aminopyridine had contrasting effects on gastrointestinal motility and gastric acid secretion. Although both tetraethylammonium and 4-aminopyridine are classic K^+ channel blockers, their sensitivity to a variety of K^+ channels is different. For example, the delayed rectifier K^+ current in canine colonic smooth muscle has been shown to consist of three distinguishable components: $I_{dK(f)}$, $I_{dK(s)}$, and $I_{dK(n)}$. $I_{dK(f)}$ is a rapidly activating 4-aminopyridine - sensitive current, $I_{dK(s)}$ is a slowly activating tetraethylammonium-sensitive current, and $I_{dK(n)}$ is a slowly activating tetraethylammonium-sensitive current with a low inactivation threshold (Carl, 1995). Since the qualitative and/or quantitative expression of ion channels expressed in each region and even within a region of the gastrointestinal tract and the sensitivity of tetraethylammonium and 4-aminopyridine for these channels are different, it is reasonable that tetraethylammonium and 4-aminopyridine may have different effects on gastrointestinal function.

We found that 4-aminopyridine increased the maximal contractile force and minimal relaxation force, and decreased the amplitude and frequency of the duodenum contraction, indicating that 4-aminopyridine induced a spastic contraction related to the inhibition of intestinal peristaltic propulsion in vivo. Likewise, studies in vitro showed that tetraethylammonium increased the maximal contractile force, the minimal relaxation force, and the amplitude of the contraction, which was coincident to the increase in gastrointestinal transit in vivo. Although there is no doubt that L-type calcium channels were the ultimate pathways by which tetraethylammonium and 4-aminopyridine influenced duodenum contraction, because both effects were abolished by verapamil, a Ca^{2+} channel blocker, their effects may be exerted through different signal transduction pathways. Boev et al. reported effects of 4-aminopyridine on the electrical and contractile activities of the fundus and antrum of the cat stomach, with 4-aminopyridine exerting its enhanced contractile effects via an increase in neurotransmitter release (low concentrations) and/or directly on the smooth muscle cell membrane (high concentrations) (Boev et al., 1985). Cheng et al. reported that 4-aminopyridine induces the release of NPY from non-adrenergic nerves to produce an atropine- and TTX-resistant contraction in the isolated jejunum of rabbits (Cheng et al., 1989). The effect of tetraethylammonium might be due to its direct inhibition of K^+ channels because trimebutine, a drug used in both hyperkinetic and hypokinetic motility disorders, is reported to inhibit the K^+ current in rabbit ileal smooth muscle cells, and the excitatory effects of trimebutine on the gastrointestinal tract may be attributable to its inhibitory action on the K^+ current (Nagasaki et al., 1993).

We found that 4-aminopyridine (2.5 mg/kg, s.c.) significantly increased the gastric acid secretion of rats, whereas Santicioli et al. reported that 4-aminopyridine reduced vagal-stimulated gastric acid secretion in the rat (Santicioli et al., 1988). This discrepancy may be due to differences in the experimental conditions.

Acknowledgements

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