THE EFFECTS OF NIFEDIPINE AND VERAPAMIL ON HIGH K^+ INDUCED CONTRACTIONS OF THE RAT INTESTINAL TRACT IN VIVO

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Abstract—The effects of intravenous nifedipine and verapamil against the contraction produced by topical application of 54 mM K^+ on the outer surface of rat duodenum, jejunum, ileum, caecum, colon and rectum have been investigated. Both drugs exhibited a descending gradient of relaxant activity with the exception of the rectum, verapamil being 1/3–1/4 as potent as nifedipine in all the test systems studied. Since these drugs at effective spasmolytic doses significantly affect heart rate and systemic blood pressure, their use as smooth muscle relaxants in intestinal disturbances would present several drawbacks.

Organic Ca^{++} entry blockers such as nifedipine and verapamil (1) have been reported to exert a relaxant effect on gut smooth muscle cells in *in vitro* (2–4) and in *in vivo* experiments (5). This, although not unequivocally proven as are their effects at the cardiac level, is commonly attributed to a direct inhibition of extracellular Ca^{++} -dependent excitation contraction coupling and to the suppression of transmembrane Ca^{++} flow-dependent spike generation (1, 6).

In the last few years, much attention has been paid to the potential use of organic Ca^{++} entry blockers to decrease the lower oesophageal sphincter pressure in patients suffering from oesophageal achalasia (7–9). Since no systematic comparative *in vivo* study concerning the smooth muscle relaxant properties of nifedipine and verapamil on the intestinal tract has so far been attempted, we decided to investigate the potential effects of these drugs on topical high- K^+ induced

contractions on different sections of rat intestinal tract (IT).

Materials and Methods

Male albino rats Wistar-Morini strain, weighing 340–360 g, were anaesthetized with subcutaneous urethane (1.2 g/kg), and the left jugular vein cannulated with a polyethylene tubing for *i.v.* injection.

Through a midline incision of the abdomen, segments of the duodenum, jejunum, ileum, caecum, colon or rectum were identified and exposed according to Morisset *et al.* (10), and silk ligatures applied at a distance of 2 cm from each other. Through a small incision, the flanged tip of a polyethylene tubing (1 mm I.D., 1.5 mm O.D.) was inserted into this pocket-like space, secured in place by means of a purse string ligature, and connected to a pressure transducer as previously described (11, 12). Warm saline soaked cotton wool swabs were laid around the exteriorized organs which were continuously washed with warm (37°C) Krebs solution of the following composition (mM): NaCl, 119;

NaHCO₃, 25; glucose, 11; KCl, 4.7; MgSO₄, 1.5; KH₂PO₄, 1.2; CaCl₂, 2.5 that was dropped from a reservoir at a rate of 20 drops/min to maintain the organs in a warm and moist condition. After a 15 min stabilization period, Krebs solution was replaced by a high-K⁺ (54 mM) Krebs solution prepared by substituting equimolar quantities of NaCl with KCl, which was delivered at a rate of 2 ml/min to produce a sustained contracture of the organs suitable for studying the relaxant effect of test substances. Topical application of the spasmogen is assumed to provide a contractile stimulus independent of the different rates of blood flow to different sections of the intestinal tract (12). Nifedipine (Bayer) or Verapamil HCl (Isoptin, Knoll) dissolved in saline were injected i.v. in increasing doses, the next dose being injected when the preceding one had reached its maximal spasmolytic effect. Nifedipine was solubilized in a small amount of absolute ethanol to obtain a 2 mg/ml stock solution from which appropriate dilutions in saline were made just before intravenous administration. Care was taken to avoid exposure of nifedipine solutions to light.

Since high K⁺ solution is known to release acetylcholine from nerve endings (13, 14), in some experiments atropine (0.5–1 mg/kg) was injected intravenously to evaluate at what extent the contractile effects of high-K⁺ are dependent upon muscarinic receptor stimulation.

The possible involvement of other neurotransmitters (catecholamine, etc.) in high-K⁺ induced contraction has not been investigated.

In preliminary experiments, the concentration response curve was obtained on rat duodenum by replacing normal Krebs with Krebs containing various concentrations of K⁺. From these results, it was found that maximal contraction of the duodenum was obtained using K⁺ 54 mM.

Spasmolytic activity was expressed as % inhibition of K⁺ induced contracture. Regression analysis was performed by the least squares method, and the inhibitory concentration (IC₅₀) calculated according to Litchfield and Wilcoxon (15).

To evaluate the effects of spasmolytic doses of nifedipine and verapamil on the cardiovascular system, male albino rats, Wistar-Morini strain, weighing 340–360 g were anesthetized with subcutaneous urethane (1.2 g/kg). The left jugular vein and femoral artery were cannulated for drug injection and mean arterial pressure (MAP) recording, respectively. Heart rate was measured by coupling the EKG signal to a Hewlett Packard 15050 A heart rate counter.

The effect of i.v. cumulative doses of nifedipine and verapamil on MAP and heart rate were recorded in two groups of six animals each and expressed as the % variation from resting values.

Statistical analysis was performed by means of the Student's *t*-test for paired data.

Results

Effects of K⁺ solution on responses of rat duodenum: Topical application of K⁺ (4.7–80 mM) to the outer surface of the rat duodenum produced a concentration dependent contraction which reached a nearly maximal response at 54 mM (Fig 1). For this reason, a K⁺ concentration of 54 mM was selected for further experiment, and this "high K⁺" produced a rapidly developing contraction of the rat jejunum, ileum, caecum, colon and rectum, the magnitude of which increased by and large according to the distance from the stomach of the different segment (Table 1).

The high-K⁺-induced contraction showed in some sections, namely, rat colon and rectum, a biphasic behaviour: a rapidly developing peak followed by a later steady level of tension which resembles the phasic

Table 1. Intraluminal pressure changes developed by different consecutive sections of rat intestinal tract stimulated by topical high-K⁺ (54 mM)

Organ	Increase in intraluminal pressure (mmHg, mean±S.E.)
Duodenum	5.1±0.6
Jejunum	6.9±0.6
Ileum	7.8±0.8
Caecum	7.1±0.8
Colon	17.5±1.5
Rectum	18.4±1.4

Each point is the mean±S.E. of 12 animals.

Fig. 1. Relationship between K⁺ concentration and changes of intraluminal pressure of the rat duodenum. Each point (○) is the mean±S.E. of 6 animals.

and tonic components produced by high-K⁺ solution commonly observed in in vitro studies (14). The steady tonic contraction produced by high-K⁺ is rapidly reverted with return to normal Krebs.

The high-K⁺ induced tonic contraction remains constant for 30 min, a period of time sufficient to determine the effect of substances.

Effects of atropine on high-K⁺ induced steady contraction: The administration of

Fig. 2. Effect of ensuing cumulative doses of intravenous nifedipine and verapamil on topical high K⁺ (54 mM) induced contracture of rat duodenum (○), jejunum (●), ileum (□), caecum (■), colon (△), and rectum (▲). Each point is the mean of 6 experiments. Vertical lines are the S.E. of the mean; when these are omitted, they are smaller than the symbol for the mean.

atropine (0.5–1 mg/kg) reduced in only a small number of animals the responses to high-K⁺, the extent of which was never more than 10% of the total response.

Table 2. Effect of nifedipine and verapamil on high-K⁺ induced contractions of different sections of rat intestine

Preparation	IC50 and 95% confidence limits ($\mu\text{g/kg}$, i.v.)			
	Nifedipine		Verapamil	
Duodenum	15	(5–35)	69	(37–129)
Jejunum	27	(15–47)	124	(73–210)
Ileum	30	(21–41)	153	(86–276)
Caecum	34	(10–112)	159	(96–265)
Colon	77	(30–196)	297	(171–514)
Rectum	24	(7–75)	133	(101–176)

Each IC50 value has been obtained from at least 6 experiments.

Effects of nifedipine and verapamil on high-K⁺ induced steady contraction: Intravenous nifedipine and verapamil (Fig. 2) produced a dose related inhibition of high-K⁺ induced steady contractions in all sections of rat IT. The calculated IC50 values for the various organs are shown in Table 2 and indicate the existence of a gradient in the relaxing properties of both drugs as the organ segments progress from the upper to the lower sections of rat IT with the exception of the rectum. In all the organs tested, verapamil was only about 1/3–1/4 as effective as nifedipine in antagonizing high-K⁺ produced contraction.

Effects of nifedipine and verapamil on heart rate and mean arterial pressure: Urethane anaesthetized rats had a resting heart rate of 304 ± 6 beats/min and a resting MAP of 106 ± 8 mmHg (mean $\pm$ S.E., $n=12$). Nifedipine in doses equal to or higher than $10 \mu\text{g/kg}$ produced a slight but still significant increase in heart rate, while verapamil in doses equal to or higher than $12.5 \mu\text{g/kg}$ produced a significant decrease in this parameter. Both drugs, produced a significant dose related decrease in MAP (Fig. 3).

Discussion

The response of in vitro smooth muscle preparations to an isotonic high-K⁺ medium produces a contraction which, after an initial peak of tension (phasic component), develops a steady level of tension (tonic component)

Fig. 3. Effect of ensuing cumulative dose of intravenous nifedipine (Δ) and verapamil ($\blacktriangle$) on heart rate (upper panel) and mean arterial pressure (lower panel) of anaesthetized rats. * $P<0.05$, ** $P<0.02$, *** $P<0.01$.

that appears to be dependent upon a trans-membrane influx of extracellular Ca^{++} through "potential sensitive calcium channels" (14, 16, 17). The tonic component of high-K⁺ induced contractions is highly sensitive to the relaxing effects of organic Ca^{++} entry blockers such as verapamil and nifedipine (1,

18). Under our experimental conditions, topical application of high-K⁺ solution produces a tonic contraction which is likely to be dependent upon mechanisms quite similar to those operating in the *in vitro* experiments. The following facts strongly support such a conclusion: a) the substitution of high-K⁺ with normal Krebs solution produces an immediate return to resting values; b) return to resting values after topical acetylcholine requires a longer time (12); and c) atropine, in doses (0.5–1 mg/kg) higher than those (0.015–0.125 mg/kg) sufficient to produce a significant blockade of cardiac cholinceptors in rats of the same age and strain (19), has only a negligible effect on topical high-K⁺ induced contraction which is highly sensitive in a dose dependent manner to low doses of Ca⁺⁺ entry blockers.

Both nifedipine and verapamil show an activity gradient in their relaxant properties when descending from the duodenum to the colon, the rectum, however, being as sensitive as the jejunum for both drugs. Other gradients have been described to occur in the gastrointestinal system concerning electrical activity (20), innervation (21), muscle response to gastrin (22), pentagastrin (23) opioid-like peptides (24) and prostanoids (25). Although no conclusion relative to the mechanism(s) underlying this phenomenon can be drawn from our data, the observation that longitudinal and circular layers of intestinal smooth muscle have a different contractile behaviour when stimulated with high-K⁺ medium (14, 26, 27), suggests that regional differences in the distribution of muscular layers might have a bearing on our results. Since contraction of intestinal smooth muscle is strongly dependent on extracellular Ca⁺⁺ (28) even when elicited with neurohormones (3, 29), it is possible that the regional differences in sensitivity to the relaxing effects of Ca⁺⁺ blockers have a functional counterpart. Under our experimental conditions, verapamil is

1/3–1/4 as effective as nifedipine in antagonizing high-K⁺ induced contraction of intestinal smooth muscle as compared to the 1/25 ratio in effectiveness at the vascular level (30, 31) and in delaying spontaneous contractions of rat urinary bladder (32).

These findings confirm the differences between organic Ca⁺⁺ entry blockers in their efficacy on different tissues which might either be attributable to the properties of the slow channels (33) or to differences in their blocking mechanisms (34, 35).

Nifedipine has been successfully employed in the treatment of oesophageal achalasia (8, 9). If decrease in effectiveness from the upper to the lower sections of the intestinal tract occurs in man similarly to that observed in rats, the successful therapy of achalasia without concomitant side effects could be ascribed to the low doses of Ca⁺⁺ entry blockers required for treatment of oesophageal spasm. However, since effective spasmolytic doses of both nifedipine and verapamil affect significantly both heart rate and systemic blood pressure, their use as smooth muscle relaxants in intestinal disturbances appears to be contraindicated.

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