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MYOELECTRIC BOWEL ACTIVITY IN ISCHEMIA / REPERFUSION DAMAGE. ROLE OF SENSORY NEURONS

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The present knowledge indicates that afferent sensory neurons (C-fibres) play an important role in the relationship between intestinal myoelectric activity (IMA) and blood flow (LDBF). The aim of this study was to evaluate the role of C-fibers in myoelectric activity of small intestine during its ischemia and reperfusion. A neurotoxin-capsaicin (CAP) was used to induce functional ablation of afferent sensory neurons. Experiments were performed on 6 groups of anesthetized rats/ In the I, II, III group of rats IMA and LDBF were recorded during 100% ischemia induced by AMA 15, 30 and 60 min total occlusion and during 60 min reperfusion period. In group V and VI, IMA and LDBF were registered after intrajejunal placement of 1% CAP. In group IV we measured effects of intraluminal instilation of CAP alone. Intraluminal placement of CAP induced an early increase in slow wave amplitude SWA and slow wave frequency SWF by $35 \pm 11\%$ and $19 \pm 10\%$ ($p < 0.05$) with the subsequent decrease in both by 25 ± 6 and $24 \pm 8\%$ ($p < 0.05$) respectively. Short 15 min lasting ischemia induced by 100% occlusion of AMA evoked only a slight increase of SWA. During reperfusion period SWA and SWF returned to the baseline values after 15 min. Total 30 min occlusion decreased SWA and SWF by 25 ± 9 and $24 \pm 6\%$ ($p < 0.05$) respectively. During reperfusion period recovery of IMA parameters to preocclusion values were slower. Intestinal hyperemia was smaller than in previous group. After 60 min lasting intestinal ischemia SWA and SWF were decreased by 58 ± 7 and $40 \pm 6\%$ ($p < 0.01$) respectively. There was no return of IMA parameters to control values. These data demonstrated that intestinal ischemia induces typical changes in the bowel myoelectric activity. These changes possess their own electrical characteristics which can be used in clinical practice for evaluation of the degree ischemically-induced intestinal injury. Capsaicin pretreatment significantly decreased SWA and SWF and LDBF in comparison with those observed in group II and III during 30 and 60 min occlusion and reperfusion period. We conclude that afferent neurons C activated during mesenteric ischemia/ reperfusion play an important role in protecting ischemic bowel viability.

INTRODUCTION

Sensory innervation of the small intestine plays an important role in the control of physiological functions of the organ including motor, secretory and absorbtive activity. This innervation plays also an important role in the modulation of intestinal local vascular responses (1—7). The sensory pathway activated by mucosal stimuli are intrinsic whereas activated by muscle stretch is mediated by extrinsic sensory neurons with cell bodies in the dorsal root ganglion. These afferent neural fibers, when stimulated can elicit intestinal vasodilation either *via* a local axon reflex, releasing vasodilator peptide neurotransmiters or *via* a long reflex which inhibits adrenergic vasomotor neurons. It has been demonstrated that afferent sensory fibers can modulate resting intestinal blood flow (1) and intestinal vascular autoregulatory responses including vascular post-stimulatory escape (5), reactive hyperemia (3) and functional hyperemia (2). Recent experimental evidence suggests that intestinal ischemia may induce typical response of the bowel myoelectric activity (8, 9). Ischemia induced electrical phaenomena could be applied in clinical practice for evaluation of the bovel viability during ischemia and reperfusion injury. Previous studies (8—10) have shown decrease of the slow waves amplitude and freguency during intestinal ischemia. Since sensory innervation of the small intestine plays an important role in the control of the myoelectric activity, the aim of the present study was to evaluate the possible role of the sensory innervation in the modulation of ischemia/ reperfusion induced intestinal myoelectric responses.

MATERIALS AND METHODS

Experiments were performed on 60 male Wistar rats weighing 250—320g. Animals were fasted, but were allowed access to water for 24h before experiments. Rats were anesthetized with intraperitoneal injections of thiopenthal (25mg/kg), intubated and ventilated with room air using a positive pressure respirator (Ugo Basile). Body temperature was maintained at 37°C by warming each animal with a heating pad controlled by a rectal thermistor. Mean systemic arterial pressure (AP) was monitored *via* a saline-filled catheter inserted into the right carotid artery and connected with a strain-gauge transducer (Statham, P231D). A midline laparotomy was performed to expose the anterior mesenteric artery (AMA). Temporal total occlusion of mesenteric blood flow was obtained with a miniature hydraulic occluder placed around the anterior mesenteric artery distal to the flow probe. Microcirculatory intestinal blood flow (LDBF) was determined by laser Doppler flowmetry (Periflux 4001 Master). A fiberooptic probe was positioned against the serosal surface of the bowel and secured outside the animal to prevent any movement of the tip of the probe. The changes in LDBF were calculated in terms of the percentage of control. Continuous recordings of AP and LDBF were made on a polygraph (Sensor Medics Dynograph model R611).

The intestinal myoelectric activity characterized by slow wave frequency (SWF) and amplitude (SWA) was recorded *via* four monopolar electrodes. Four silver monopolar electrodes were implanted on the serosal surface of the small bowel. Electrode number one was localized in first part of the jejunum and the following three electrodes were implanted 5, 10 and 15 cm distally. IMA was analyzed by the computer program (Gascan-Proster).

After completing the surgical preparation, LDBF, AP and IMA were allowed to stabilize for 30 min, then one of experimental protocols was initiated. In each protocol, a group of 10 rats was studied. Rats in group I, II and III were only vehicle treated.

In group I of rats we observed the IMA and LDBF responses to 15 min lasting 100% occlusion of AMA and 60 min post-occlusion reperfusion period.

In group II of rats the jejunal myoelectric response and LDBF to 30 min period of ischemia and 60 min reperfusion was studied.

In group III of rats IMA and LDBF were studied in animals underwent 60 minutes of AMA occlusion followed by 60 minutes of reperfusion.

In group IV of rats the effects of acute intrajejunal instillation of capsaicin on IMA and LDBF were examined.

Capsaicin (Fluka) was administered as a 1% solution (10% ethanol, 10% Tween 80, and 80% 0.9% NaCl).

For this purpose, a sillicone cannula was inserted into the proximal part of the jejunum through a small incission located 2 cm distal to the pylorus, then 1 ml of capsaicin solution was injected slowly into the intestinal lumen.

The responses of LDBF and IMA to capsaicin were quantified by determining the percentage change in LDBF, SWA and SWF during the early response as well a later response in comparison with the control values of measured parameters.

In group V of rats IMA and LDBF response to 30 minutes of ischemic period followed by one hour reperfusion was examined after acute pretreatment with capsaicin. The IMA and LDBF response in this group was compared statistically with the response observed in group II.

In group VI of rats IMA and LDBF response to 60 minute of ischemic period followed by 60 minutes of reperfusion was examined after pretreatment with capsaicin. The IMA and LDBF responses in this group were compared with the changes observed in group III.

All experimental data were presented as means $\pm$ SE. The significance of changes in measured values from control were determined using the two-tailed student's t-test for either grouped or paired data with a confidence limit of less than 5%.

RESULTS

In the six experimental designs, the mean basal LDBF was 286 ± 24 PU under control conditions. The mean basal AP range was 110—123 mmHg. Control SWF was 30 ± 2 cycles/min and SWA was 0.3 ± 0.08 milivolts.

In group I SWA increased significantly by $25 \pm 7\%$ ($p < 0.05$) and SWF did not change significantly by 15 min of ischemia. During reperfusion period SWA returned to baseline values after 15 minutes. Following release from arterial occlusion LDBF increased rapidly $30 \pm 6\%$ ($p < 0.05$) above control preocclusion level and then returned gradually to the control values. At the end of the reperfusion period all measured parameters did not vary from their control values.

In group II SWA decreased by $25 \pm 9\%$ ($p < 0.05$) and SWF decreased by $24 \pm 6\%$ ($p < 0.05$) at 30 minutes of intestinal ischemia. During reperfusion period, recovery of SWA and SWF values to the preocclusion level was observed but the rate of the return of IMA to the baseline values was slower than that seen in the group I. Following release from arterial occlusion intestinal hyperemia was seen but in a lesser degree than in group I. Both electrical and circulatory parameters returned to baseline values at the end of the reperfusion period (*Fig. 2*).
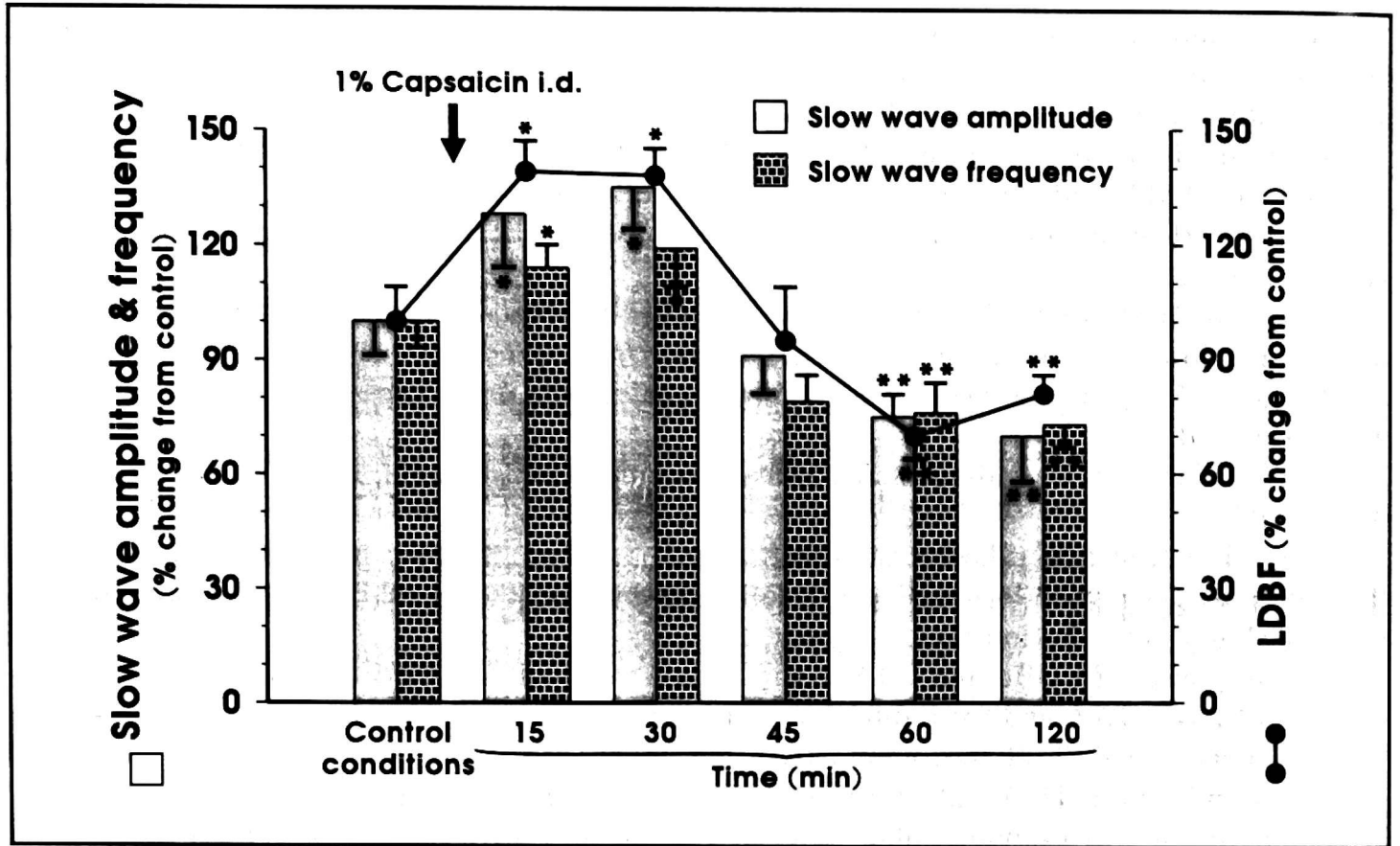
Fig. 1. Effects of intrajejunal instillation of capsaicin on intestinal microciculatory blood flow, slow wave amplitude and frequency. *significant increase above the control value. **significant decrease below the control value.
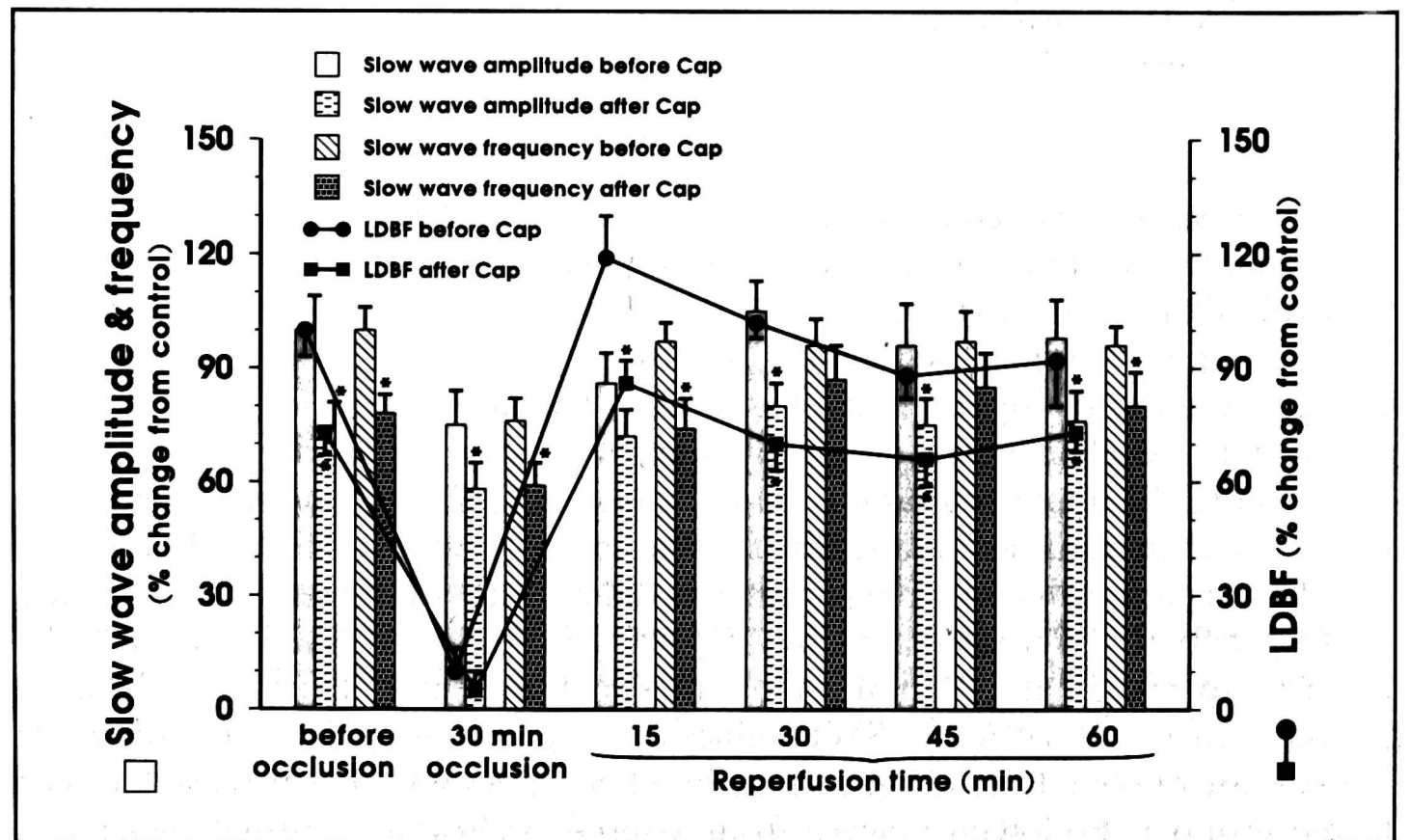

Fig. 2 Effects of 30 minutes occlusion of AMA followed by 60 minutes reperfusion period on LDBF, SWA and SWF before (group II) and after pretreatment with capsaicin (group V). *significant decrease below the precapsaicin values.

In group III at the end of 60 minutes intestinal ischemia SWA was decreased by $58 \pm 7\%$ and SWF by $40 \pm 6\%$ ($p < 0.01$). After arterial occlusion was ended intestinal microcirculatory blood flow reached $30 \pm 5\%$ ($p < 0.05$) of its control value only.There was no return of measured electrical and circulatory parameters to control values. (*Fig. 3*)
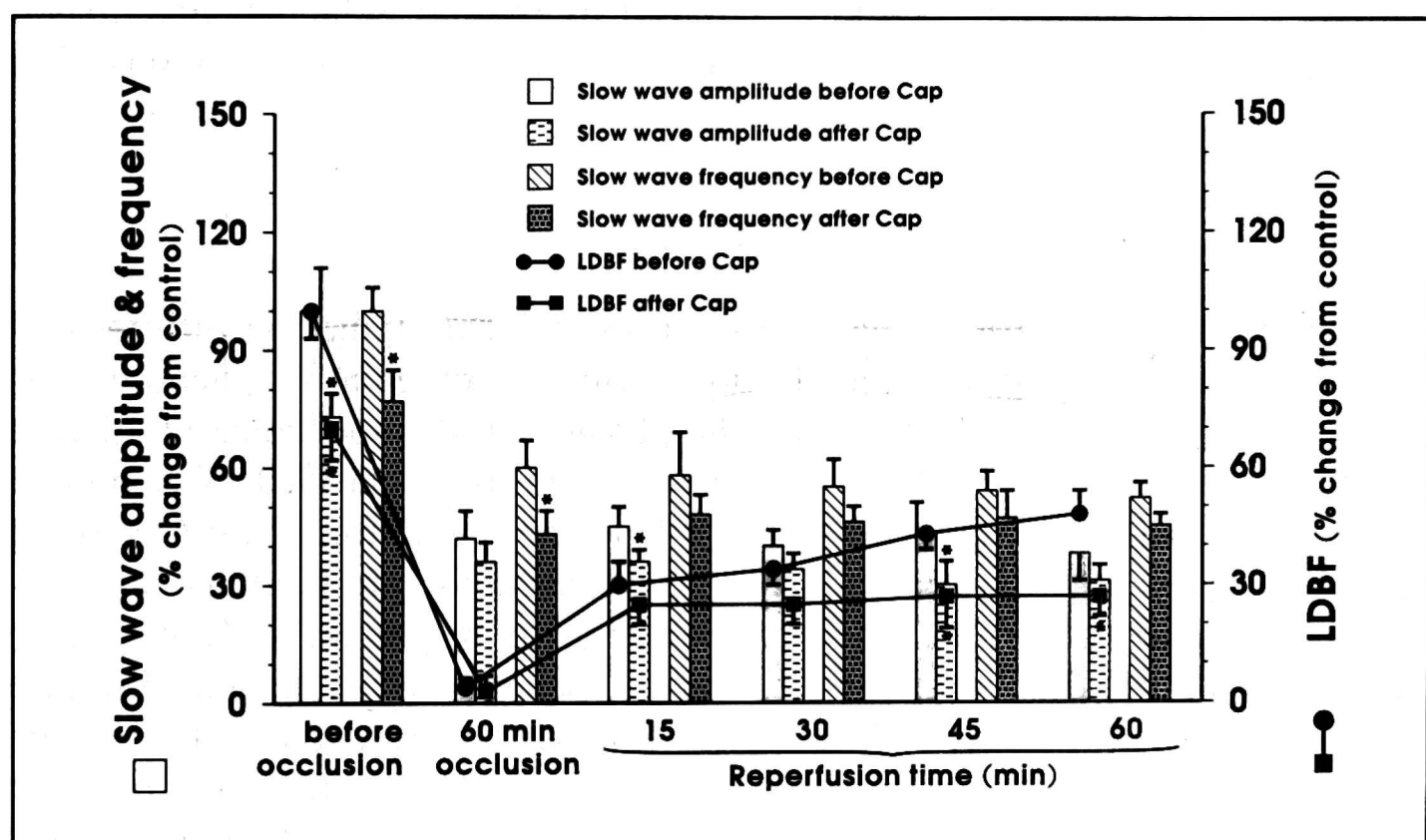

Fig. 3. Efects of 60 minutes occlusion of AMA and subsequent 60 minutes reperfusion on LDBF, SWA and SWF before (group III) and after pretreatment with capsaicin (group VI). * significant decrease below the precapsaicin values.

In group IV the early and late effects of acute intraluminal application of capsaicin were studied. Immediately after application of capsaicin, an increase in LDBF was observed. The maximal hyperemic response was observed at 15 min after onset of drug administration, when BF was increased by ($39 \pm 8\%$ ($p < 0.01$). Subsequently, a slow recovery of LDBF towards the control value was observed. This vasodilatory response to capsaicin had a latency of up to 30 min and was followed by a long period of vasoconstriction. The new steady-state LDBF was obtained at 60 min after the onset of capsaicin administration. At that time LDBF was decreased by $30 \pm 6\%$ from the control value. Intrajejunal capsaicin induced also an early increase in SWA by $35 \pm 11\%$ ($p < 0.05$) and in SWF by $19 \pm 10\%$ ($p < 0.05$). Subsequently, there was a decrease in SWA by $25 \pm 6\%$ ($p < 0.05$) and in SWF by $24 \pm 8\%$ ($p < 0.05$) in comparison with the precapsaicin control values (*Fig. 1*).

In group V rats pretreated with capsaicin 60 min before the experiments, SWA decreased $42 \pm 7\%$ ($p < 0.05$) and SWF decreased $41 \pm 6\%$ ($p < 0.05$) by 30 minutes of intestinal ischemia. During reperfusion period only not significant

tendency of recovery of SWA and SWF towards the control level was observed. Following release from arterial occlusion there was no return of LDBF to the control value. Electrical and circulatory parameters in this group were significant different in comparison with group II rats (*Fig. 2*).

In group VI rats pretreated with capsaicin depression of the IMA parameters and reduction of LDBF induced by 60 min ischemia/ reperfusion parameters was significantly potentiated in comparison with those observed in group III (*Fig. 3*).

DISCUSSION

The physiological role of afferent C-fibres in local regulation of intestinal blood flow at basal conditions and during vascular autoregulatory phenomena is well recognized (1—7, 11). However, the physiological importance of afferent C-fibers in the regulation of IMA responses remains to be clarified. We assumed that visceral C-fibers might also play a role in the regulation of IMA at basal conditions and during ischemia/ reperfusion induced intestinal damage.

The intestinal microvascular response observed in the present study after acute application of capsaicin consisted of an early hyperemia with a maximal increase in LDBF 15 min after onset of drug application, followed by a gradual late decline of LDBF below the control value. The late response of LDBF was stable and persistent at 60 and 120 min. In every case, parallel changes were observed in IMA, which suggest that a close relationship exists between LDBF and IMA.

The characteristics of the intestinal microcirculatory response, which we observed after acute capsaicin treatment are consistent with previous reports that acute intraluminal capsaicin prompted a marked intestinal hyperemia in rats (2, 4, 12). The electrical characteristics of capsaicin-induced IMA observed in the present study indicate that early stimulation of IMA was present. This early effect appeared 15 min after onset of capsaicin instillation and lasted 30 min. Initial stimulatory effects could by attributed to release of SP and neurokinin A (NKA) which are known to stimulate IMA. The excitatory effect of capsaicin on IMA was followed by a late decline of IMA below the control level. The current findings that capsaicin inhibits IMA are similar to previous reports of others (13—16).

It has been demonstrated that acute periarterial capsaicin induces a vasodilation secondary to a local release of transmitter substances from sensory nerve endings within the intestinal wall. However, intraluminal capsaicin may stimulate release of these transmitters *via* a local axon reflex in the intestinal mucosa (12—14, 17). In rat gut the early vascular and IMA responses to acute capsaicin appear attributable to release of vasodilator peptides such as VIP, SP and CGRP. Vasodilatory neurokinins may also be released from sensory neurons after acute activation with capsaicin (2, 11—14, 18).

Upon release some of these peptides cause nonadrenergic and noncholinergic vasodilation of the intestinal microcirculation. The persistent intestinal vasoconstriction and inhibition of IMA which we observed after acute capsaicin, may be the result of the depletion of vasodilatory neuropeptides.

This capsaicin-induced vasoconstrictor response and inhibitory effect on IMA response also indicates that tonic release of vasoactive peptides participates in the control of the normal intestinal vascular tone and IMA.

Our findings on the acute effects of capsaicin on IMA are in agreement with other reports (15). However, in our study we observed additionally capsaicin-induced initial increase in intestinal myoelectric activity. Moreover, Shea-Donohue et al. has reported that intraluminal capsaicin did not alter intestinal myoelectric activity but induced inhibition of stimulatory action of ricin (19). These late effects were attributed to inhibition of LTC_4 and PGE_2 synthesis by capsaicin. Our observation that IMA was significantly diminished by depletion of sensory neuropeptides with capsaicin suggests that the mentioned neurotransmiter peptides participate in the mediation of basic mechanism of small intestinal electric activity.

The physiological importance of the intestinal microcirculation to maintain tissue metabolic needs and integrity is well recognized. Intestinal myoelectric activity appears to be associated with blood flow. Ischemic hypoxia induces characteristic changes in IMA which can be applied in experimental and clinical practice for evaluation of the bowel viability.

Electromyogram of the intestinal muscle contains two electrical phaenomena, one recurring at regular intervals called slow waves and second spike potentials which occur in random fashion on the plateau phase of the slow waves. Slow waves are phase locked with cephalad phase lag and are metabolic dependent. The enteric nervous system that is governing electrical and mechanical acitivity consists of sensory, inter and motoneurons. Cajal's cells of myenteric, plexus acts as a pacesetter for IMA. Sensory neurons are specialized for detecting thermal, chemical and mechanical stimuli and transform the information into action potential code. The main mechanical event which is responsible for gastrointestinal motility is stereotyped contractions called peristaltic reflex. It could be elicited by muscle stretch and by stimulation of the mucosal receptors. The sensory pathways for activation of mucosal receptors are wholy intrinsic therefore more sensitive to bowel ischemia than activated by muscle stretch. However sensory fibers C are necessary for activation of both.

Our present study also shows that intestinal ischemia and post-ischemia reperfusion is characterized by decrease of SWA and SWF, except short period of ischemia. These results clearly shows that IMA in the rats small bowel can be disrupted during local intestinal ischemia. Short lasting ischemia induces only transient disturbances in IMA, whereas longer than 30 min ischemia induces pronounced decrease in SWA, SWF and LDBF observed during whole

reperfusion period. In animals pretreated with capsaicin the intestinal electrical and circulatory injury induced by ischemia and reperfusion was significantly aggravated (*Fig 2 i 3*).

Previous studies have presented evidence to support a physiological role for capsaicin-sensitive nerves as mediators of reflex and autoregulatory events in the circulation of rat gut (1—8, 20—22). Results of our current investigation indicate that these nerves also modulate intestinal myoelectric activity at basal conditions and during ischemia/ reperfusion phenomenon. Our observation that ischemia/ reperfusion- induced IMA and circulatory injury were significantly potentiated by depletion of sensory neuropeptides with capsaicin suggest that beforementioned neurotransmitter peptides participate in the mediation of intestinal myoelectric and microcirculatory protection against ischemic hypoxia. On the present stage of research, although the mechanism of sensory neurones and/ or peptides-induced intestinal protection remains unclarified. It is possible that the protective activity of sensory neurons is related to their primary influence on pacemakers cell (13), and secondary to the modulation of intestinal tissue oxygenation (11).

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