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ROLE OF CALCITONIN GENE RELATED PEPTIDE IN THE MODULATION OF INTESTINAL CIRCULATORY, METABOLIC AND MYOELECTRIC ACTIVITY DURING ISCHEMIA/REPERFUSION

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Calcitonin gene related peptide (CGRP) is widely distributed in the myenteric neurons along GI tract. Biological effects of CGRP on gastrointestinal tract include increase in the intestinal blood flow, relaxation of the smooth muscle and slight increase in the slow wave amplitude (SWA) with no effect on frequency (SWF) of intestinal myoelectric activity (IMA). The aim of this study was to evaluate the possible protective effects of endo- and exogenous CGRP against ischemia/reperfusion (I/R) induced bowel injury in rats. Experiments were performed on 30 fasted anesthetized Wistar rats. Systemic arterial blood pressure (AP), mesenteric blood flow (MBF) and microcirculatory intestinal blood flow (LDBF) were measured. Oxygen consumption (VO_2) was estimated from MBF and mesenteric AVO_2 difference. IMA was recorded by 4 monopolar electrodes in proximal jejunum and analyzed using computer program with Fourier analysis of SWF. Control ischemia induced by 30 min total occlusion of anterior mesenteric artery (AMA) reduced SWA by $25 \pm 5\%$ and SWF by $24 \pm 4\%$. At the end of 60 minute reperfusion period SWA and SWF values were restored to control values but SWF showed instability. At the 15th minute of reperfusion period MBF, LDBF and VO_2 increased to 109 ± 6 , 119 ± 11 and $120 \pm 7\%$ of control values respectively. Infusion of exogenous CGRP ($0.16 \mu\text{g}/\text{kg}/\text{min}$ i.a.) increased MBF, LDBF and VO_2 by 26 ± 6 , 31 ± 9 and $20 \pm 4\%$ respectively in comparison to control values. In the same experimental group SWA increase of 14% was observed with not significant changes in SWF. In the group where CGRP was administered before and during 30 min period of intestinal ischemia MBF, LDBF and VO_2 values at 15th minute of reperfusion were significantly higher by 24 ± 6 , 32 ± 7 and $17 \pm 5\%$ respectively in comparison to the values observed in the control reperfusion. In that group SWA values were restored to preocclusion values at 15th minute of reperfusion yet and SWF showed much more stability. Infusion of CGRP receptor antagonist (CGRP 8-37) reduced MBF, LDBF and VO_2 by 12 ± 7 , 14 ± 8 and $11 \pm 6\%$ respectively (differences not significant versus control). In I/R group when CGRP 8-37 was given hemodynamic parameters (during reperfusion) were significantly lower and IMA parameters were not restored to preocclusion values. We conclude, that endogenous and exogenous CGRP attenuate circulatory parameters of the small intestine during ischemia and reperfusion. A direct correlation exists between hemodynamic, metabolic and myoelectric effects of CGRP.

Key words: CGRP, ischemia/reperfusion, intestinal myoelectric activity, blood flow, oxygen uptake.

INTRODUCTION

The neurogenic tone of the small intestinal blood vessels is mainly determined by extrinsic autonomic innervation and by intrinsic nerves of the enteric system (1—4). The extrinsic innervation of intestinal vessels is primarily adrenergic. However, there are examples of nonadrenergic and noncholinergic innervation of the mesenteric vasculature. In addition, it has been shown that the intestinal vessels are innervated by visceral sensory, afferent nerves (C-fibers). These unmyelinated C-fibers contain a variety of well-recognized peptides, such as substance P, vasoactive intestinal polypeptide, CCK, somatostatin and calcitonin gene related peptide (CGRP) (4). These peripheral peptide-containing fibers appear to play an important role in the transmission of sensory impulses in intestinal reflexes, in the control of intestinal motor, secretory and absorptive functions. It has also been demonstrated that C-fibers can control tonically resting, intestinal blood flow and vascular autoregulatory responses such as reactive hyperemia, functional hyperemia and autoregulatory escape (1—5).

Previous studies (6—11) have shown the physiological importance of C-fibers in the regulation of intestinal motor and myoelectric activity (IMA) at normal conditions and during of ischemia/reperfusion induced intestinal myoelectric responses.

Since CGRP is one of the most important sensory peptide present in the C-fibers we assumed that this peptide might also play a role in the control of IMA and metabolic activity at basal conditions and during ischemia/reperfusion induced intestinal damage. Therefore, the aim of the present study was to evaluate the possible protective effects of endogenous and exogenous CGRP against ischemia/reperfusion induced bowel injury in rats.

MATERIALS AND METHODS

The experiments were performed on 75 male Wistar rats weighing 270—330 g. Animals were fasted but were allowed access to water for 24 hrs before the experiments. Rats were anesthetized with intraperitoneal injections of thiopental (25 mg/kg) (Biovit, Puławy Poland). Animals were intubated and artificially ventilated with room air using a positive pressure respirator (UGO Basile, Italy). Body temperature was maintained at 37°C by warming each animal with heating pad controlled by rectal thermistor (Fine Science Tools). Mean systemic arterial pressure (AP) was monitored via saline-filled catheter inserted into the right carotid artery and connected to a strain-gauge transducer (Statham, P231 D). A midline laparotomy was performed to expose the anterior mesenteric artery (AMA). Total blood flow in the AMA (MBF) was measured using an ultrasonic flow probe of 1.0 mm internal diameter (IRS), which was positioned around the AMA and connected with a blood flowmeter (Altron T206, USA).

Temporal total occlusion of mesenteric blood flow was obtained with a miniature hydraulic occluder placed around the anterior mesenteric artery distal to flow probe. Microcirculatory intestinal blood flow (LDBF) was determined by laser Doppler flowmetry (Periflux 4001 Master, Sweden). A fiberoptic probe was positioned against the serosal surface of the bowel and was secured outside the animal to prevent any movement of the probe tip. The changes in LDBF were

calculated in terms of the percentage of control. Continuous recordings of AP, MBF and LDBF were made on a polygraph (Sensor Medics Dynograph model R611).

One femoral artery was cannulated for sampling arterial blood and a side branch of the mesenteric vein was also cannulated to sample the venous blood draining the small intestine under study. Both arterial and mesenteric venous blood samples were used to determine arteriovenous oxygen content difference (AVO_2) using a spectrophotometric oxygen content analyzer (AVOXIMETER 1000E, Avox System, USA). Intestinal oxygen uptake (VO_2) was calculated as the product of the MBF and AVO_2 and was expressed in ml O_2 ml/min.

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. One electrode was localized in the first part of the jejunum and the following three electrodes were implanted 5, 10 and 15 cm distally to the first one. IMA was analyzed by the computer program (Monitor X).

After completing the surgical preparation MBF, 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.

In group I of rats we observed the MBF, LDBF, VO_2 and IMA responses to a 30 min total occlusion of AMA and 60 min post occlusion reperfusion period.

In group II of rats the effects of infusion of exogenous CGRP (0.16 $\mu\text{g}/\text{kg}/\text{min}$) infusion into the abdominal aorta (i.a.) on IMA, MBF, LDBF and VO_2 were studied in normal rats.

In group III of rats the electric, circulatory and metabolic parameters above were examined in animals underwent 30 minutes of total AMA occlusion followed by a 60 min of reperfusion during infusion of exogenous CGRP (0.16 $\mu\text{g}/\text{kg}/\text{min}$ into abdominal aorta).

In group IV of rats the effects of CGRP receptor blocker (CGRP₈₋₃₇) on IMA MBF, LDBF and VO_2 were examined in normal animals.

In group V of rats MBF, LDBF, VO_2 and IMA were studied during ischemia reperfusion period after pretreatment of animals with CGRP receptor antagonist CGRP₈₋₃₇ (10 $\mu\text{g}/\text{kg}$ i.v.) (Sigma).

All experimental data are presented as means SE. The significance of changes in the measured values from control were determined using the two-tailed student's test for either grouped or paired data with a confidence limit of less than 5%.

RESULTS

In the experimental groups, the mean basal MBF was 12 ± 3 ml/min, LDBF was 295 ± 27 PU under control conditions. The mean basal VO_2 was 0.54 ± 0.06 ml/min and AP ranged between 105–120 mmHg. Control SWF was 0.56 ± 0.049 Hz and SWA in millivolts (mV) was 0.31 ± 0.03 .

In group I at the end of 30-min ischemia, SWA decreased significantly by $25 \pm 5\%$ ($p < 0.05$) and SWF decreased by $24 \pm 4\%$ ($p < 0.05$). At the end of 60 minute reperfusion period both SWA and SWF values were restored to control values but SWF showed instability. Following release from arterial occlusion, MBF increased by $9 \pm 6\%$ NS.

LDBF increased by $19 \pm 11\%$ ($p < 0.05$) and VO_2 increased to $120 \pm 7\%$ ($p < 0.05$) of control at the 15th minute of reperfusion. At the end of reperfusion period (60th minute) both circulatory parameters were reduced — MBF by $20 \pm 10\%$ ($p < 0.05$) and LDBF by $12 \pm 4\%$ ($p < 0.05$) in comparison to control. At that time VO_2 was significantly reduced by $45 \pm 8\%$ ($p < 0.05$) (Fig. 1–3).

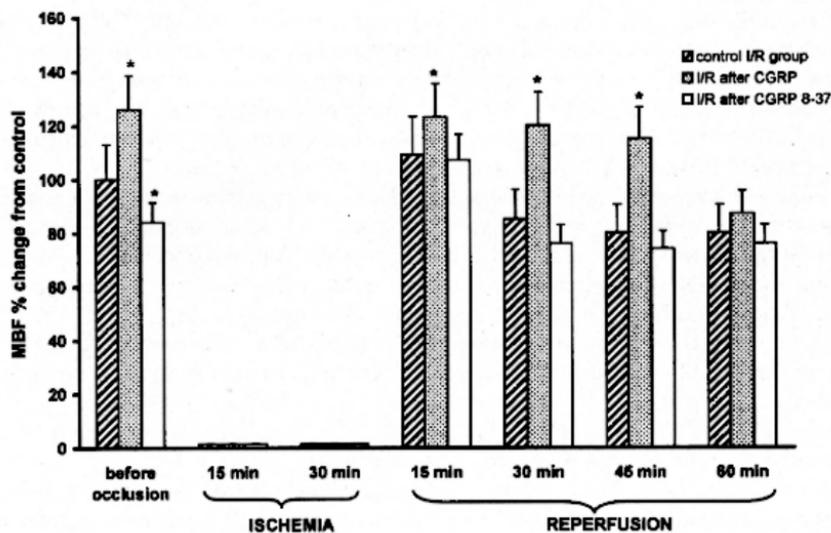


Fig. 1. Effect of 30 minutes occlusion of AMA and subsequent 60 minutes reperfusion on MBF before and after pretreatment with CGRP or CGRP 8—37.

* significantly different in comparison to I/R group at the same time.

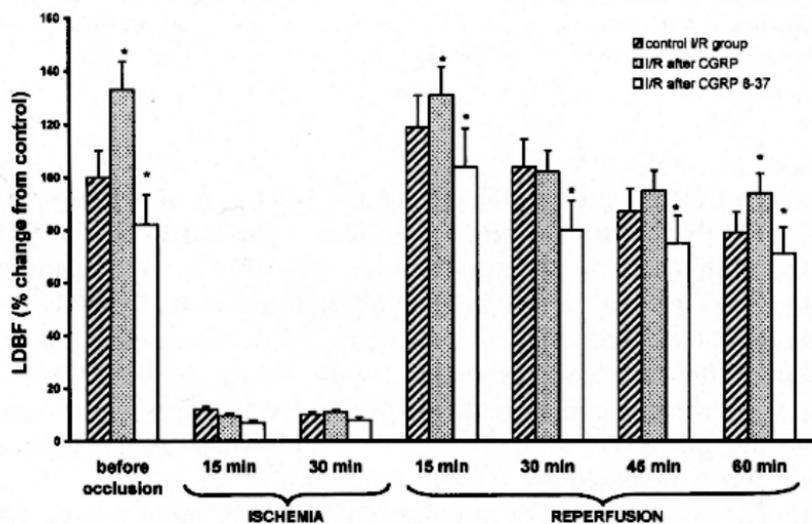


Fig. 2. Effects of 30 minutes occlusion of AMA followed by 60 minutes reperfusion period on LDBF before and after pretreatment with CGRP or CGRP 8—37.

* significantly different in comparison to I/R group at the same time.

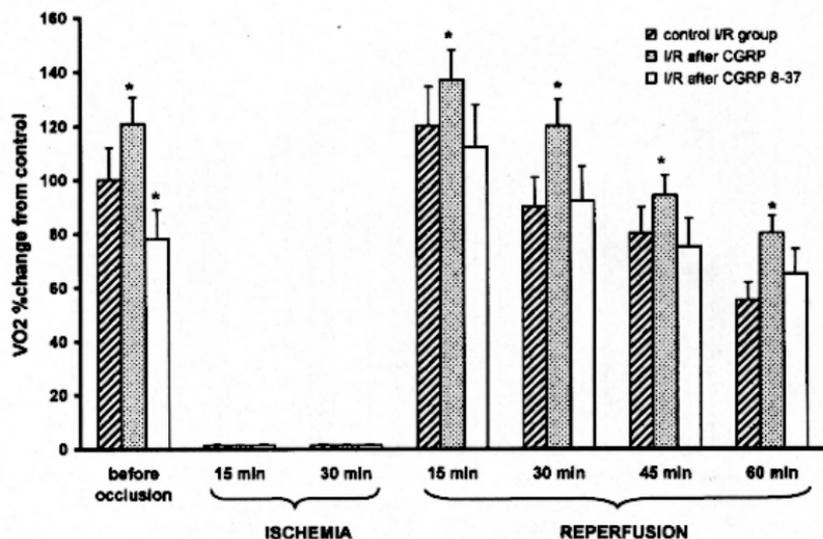


Fig. 3. Effects of 30 minutes occlusion of IMA followed by 60 minutes reperfusion on VO_2 before after pretreatment with CGRP or CGRP 8—37.

* significantly different in comparison to I/R group at the same time.

In group II, infusion of CGRP increased MBF, LDBF and VO_2 by $26 \pm 6\%$ ($p < 0.005$), $31 \pm 9\%$ ($p < 0.05$) and $20 \pm 4\%$ ($p < 0.01$), respectively in comparison to control values. CGRP infusion in that group was accompanied by 11%, non statistic reduction of arterial pressure. In the same experimental group SWA increase by $14 \pm 3\%$ ($p < 0.05$) was observed with not significant changes in SWF (Fig. 4, 5).

In group III where CGRP was administered before and during intestinal ischemia/reperfusion MBF, LDBF and VO_2 values during preocclusion time were significantly higher by $24 \pm 6\%$ ($p < 0.01$), $32 \pm 7\%$ ($p < 0.05$) and $17 \pm 5\%$ ($p < 0.05$), respectively in comparison to group I. However, in that group a rapid restoration of SWA to preocclusion values was observed in the 15th minute of reperfusion.

SWF showed much more stability and at the end of reperfusion was not different to that observed before clamping AMA. In that group a significant elevation of MBF values by 23 ± 6 , $20 \pm 6\%$ ($p < 0.005$) and by $15 \pm 4\%$ ($p < 0.05$), respectively was observed at the 15th, 30th and 45th minute of reperfusion in comparison to corresponding MBF values in the group I. That was accompanied by a marked increase in VO_2 values beginning from the 15th min of reperfusion. VO_2 values were higher by $17 \pm 3\%$, 20% ($p < 0.001$), 14% ($p < 0.03$) and 25% ($p < 0.001$) at the 15th, 30th, 45th and 60th min of reperfusion respectively in comparison to the results observed in group I.

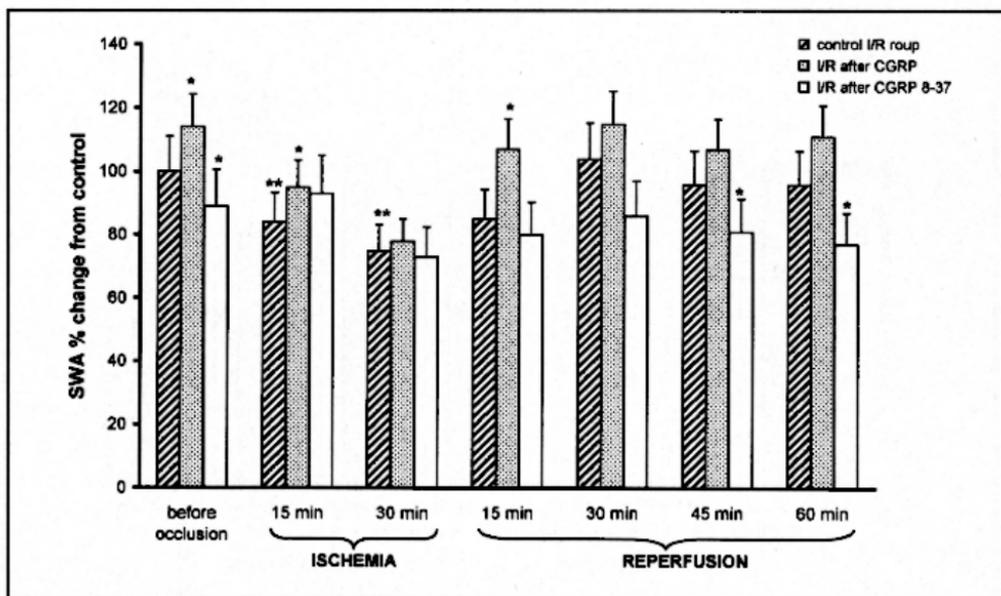


Fig. 4. Slow wave amplitude (SWA) during ischemia and reperfusion period (30 and 60 min) before and after CGRP or after CGRP 8–37.

* significantly different in comparison to I/R group at the same time.

** significantly different in comparison to SWA before occlusion.

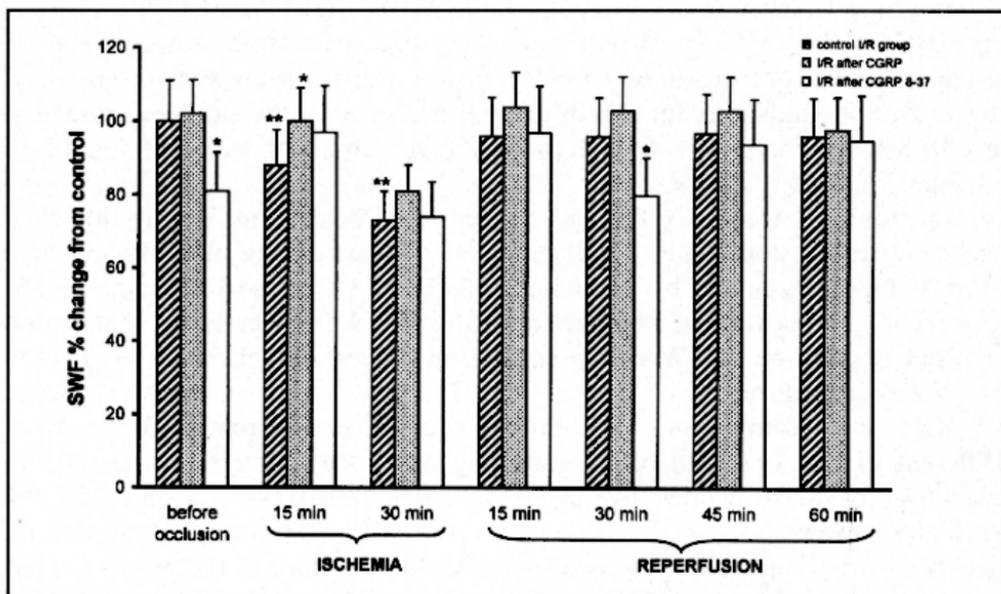


Fig. 5. Slow wave frequency SWF during ischemia and reperfusion period (30 and 60 min) after CGRP or after CGRP 8–37.

* significantly different in comparison to I/R group at the same time.

** significantly different in comparison to SWF before occlusion.

In group IV pretreatment with CGRP receptor antagonist (CGRP₈₋₃₇) reduced MBF, LDBF and VO₂ by 16±4% (p<0.05), 18±6% (p<0.02) and 22±6% (p<0.005), respectively. Significant change was observed both in the SWA and SWF values 21±6 (p<0.03) and 18±4% (p<0.04) respectively.

In group V when (CGRP₈₋₃₇) was given before occlusion and reperfusion LDBF at the 15th min of reperfusion was significantly lower by 15% (p<0.01) in comparison to the values observed in group I. No significant changes of MBF and VO₂ values were observed at that time. But in the next 45 min of reperfusion a significant reduction in LDBF and MBF was observed. In 30th, 45th and 60th min LDBF values in this group were lower by 22±5% (p<0.005), 11±3% (p<0.05) and 14±5% (p<0.05) respectively in comparison to corresponding values observed in group I. During the occlusion time there was no difference in manner of reduction of SWA and SWF values. In the reperfusion time there was no restitution of SWA to preocclusive values and SWF was still reduced by 19% (p<0.04) at the 30th min of reperfusion in comparison to SWF value in group I. At the end of reperfusion SWF values were not different from control and from those observed in group I but SWA value was significantly reduced by 20±4% (p<0.05).

DISCUSSION

Previous studies have presented the evidence supporting a physiological role of afferent C-fibers in local regulation of intestinal blood flow and oxygen uptake at basal conditions. The present knowledge indicates that C-fibers play an important role in the modulation of intestinal myoelectric activity at rest and during intestinal ischemia/reperfusion phenomenon (12, 13). Our earlier findings that I/R induced IMA and circulatory injury were significantly potentiated by depletion of sensory neuropeptides with capsaicin indicate that beforementioned neurotransmitter peptides participate in the mediation of intestinal myoelectric and circulatory protection against ischemia/reperfusion-induced injury (12, 14—19).

In the rat gut the early vascular and IMA responses to acute capsaicin appear attributable to release vasodilator peptides such as VIP, SP and CGRP from C-fibers.

In the present study we have assessed the role of endogenous and exogenous CGRP in the control of intestinal circulation, oxygenation of intestinal tissues and IMA at basal condition and during ischemia/reperfusion-induced intestinal damage. The characteristics of the changes in the intestinal and systemic circulation which appeared after i.a. infusion of CGRP are consistent with previous reports (5, 12—14). We found that CGRP elicited vasodilatory response from AMA and the

microcirculation (LDBF). We also found that CGRP evoked significant increase in VO_2 . The microcirculatory structures of which vasoactive factors act in the intestinal circulation are the arterioles which regulate resistance to the total blood flow through the intestinal wall and the precapillary sphincters which regulate the blood flow through the nutrient circulation. Since oxygen significantly exchanges only across the capillary endothelium, an increased uptake of oxygen ensues when capillary blood flow is increased. An accepted measurement of the nutrient circulation can be obtained using the local laser Doppler flowmetry. In the present studies CGRP increased intestinal macro- and microcirculatory blood flow. The observed increase in VO_2 could be due to either a direct metabolic effect of CGRP or simply to opening of the underperfused capillaries in the intestinal microcirculation at basal conditions. During hyperemic response CGRP reduced intestinal oxygen extraction and increased intestinal blood flow more proportionally than the increase in intestinal oxygen uptake. The last findings are in agreement with previous reports from our laboratory showing that acute activation of C-fibers and numerous endo- and exogenous vasodilators evoke the same pattern of responses in the mesenteric circulation (5).

In the present study we also found that the blockade of CGRP receptors (17, 18) evoked a significant decrease in intestinal macro- and microcirculatory blood flow. The blockade of CGRP receptors evoked a significant decrease in intestinal oxygen consumption. This combined with decreasing intestinal blood flow could be due either to a direct metabolic effect, or to a decrease in blood flow through the nutrient portion of the intestinal microcirculation. Our data suggests that inhibition of CGRP receptors induced indiscriminate contraction of arteriolar and precapillary sphincteric smooth muscle thereby reducing total blood flow by constricting arterioles and reducing the nutrient circulation by dosing capillaries.

The electrical characteristics of CGRP-induced IMA observed in the present study indicate that stimulation of IMA was present. Our observation that IMA was significantly diminished by blockade of CGRP receptors suggests that CGRP participates in the modulation of basic mechanism of small intestinal electric activity.

The physiological importance of the intestinal blood flow to maintain tissue metabolic need and integrity is well recognized. IMA appears to be closely associated with organ blood flow. Intestinal tissues ischemic hypoxia induces typical changes in IMA which can be applied in experimental and clinical practice for evaluation of bowel viability (19, 20).

The present study shows that total intestinal ischemia lasting 30 minutes is characterized by decrease in SWA and SWF at control conditions. In the reperfusion period both SWA and SWF return to the preocclusion values. The results above clearly show that IMA in the rat small bowel can be disrupted

during local intestinal ischemia. As was demonstrated in our studies short lasting ischemia induces only transient changes in IMA. In animals pretreated with CGRP the intestinal electrical and circulatory injury responses induced by ischemia and reperfusion were significantly diminished. We also found that the blockade of CGRP receptors evoked a significant potentiation of ischemia/reperfusion-induced IMA and circulatory injury (Fig. 4, 5).

Above results suggest that endogenous neurotransmitter peptide such as CGRP participate tonically in the modulation of intestinal electric activity, blood flow and oxygenation of intestinal tissues. Exogenous CGRP when administered directly into the mesenteric circulation diminishes the degree of the IMA, circulatory and metabolic injury induced by ischemia/reperfusion.

In summary, the findings of the present investigation support the concept that CGRP is a vasorelaxing factor in the intestinal macro and microcirculation and regulator of the intestinal tissues oxygenation during physiological and pathological conditions. It is possible that observed protective activity of CGRP is related to its effect on intestinal tissue metabolism.

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