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Altered responsiveness of the guinea-pig isolated ileum to smooth muscle stimulants and to electrical stimulation after *in situ* ischemia

*,[1]Rodolfo Rodriguez, [1]Rosa Ventura-Martinez, [1]Jacinto Santiago-Mejia, [1]Maria R. Avila-Costa & [1]Teresa I. Fortoul

[1]Departments of Pharmacology and Cellular and Tisular Biology, School of Medicine, National University of Mexico, Mexico City C.P. 04510, Mexico

1 We evaluated changes in contractility of the guinea-pig isolated ileum, using intact segments and myenteric plexus-longitudinal muscle (MPLM) preparations, after several times (5–160 min) of ischemia *in situ*.

2 Intestinal ischemia was produced by clamping the superior mesenteric artery. Ischemic and nonischemic segments, obtained from the same guinea-pig, were mounted in organ baths containing Krebs-bicarbonate (K-B) solution, maintained at 37°C and gassed with 95% O_2/5% CO_2. The preparations were allowed to equilibrate for 60 min under continuous superfusion of warm K-B solution and then electrically stimulated at 40 V (0.3 Hz, 3.0 ms). Thereafter, complete noncumulative concentration–response curves were constructed for acetylcholine (ACh), histamine (HIS), potassium chloride (KCl), and barium chloride ($BaCl_2$). Mean E_{max} (maximal response) values were calculated for each drug.

3 Our study shows that alterations of chemically and electrically evoked contractions are dependent on ischemic periods. It also demonstrates that contractile responses of ischemic tissues to neurogenic stimulation decreases earlier and to a significantly greater extent than the non-nerve mediated responses of the intestinal smooth muscle. Contractile responses to smooth muscle stimulants were all similarly affected by ischemia. Electron microscopy images indicated necrotic neuronal death. The decrease in reactivity of ischemic tissues to electrical stimulation was ameliorated by dexrazoxane, an antioxidant agent.

4 We consider the guinea-pig isolated ileum as a useful model system to study the processes involved in neuronal ischemia, and we propose that the reduction in maximal responses to electrical stimulation is a useful parameter to study neuroprotection.
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Abbreviations: ACh, acetylcholine; $BaCl_2$, barium chloride; CNS, central nervous system; ENS, enteric nervous system; HIS, histamine; I/S, ischemia/superfusion; K-B solution, Krebs-bicarbonate solution; MPLM, myenteric plexus-longitudinal muscle preparation; KCl, potassium chloride; SCAS, sequential common carotid artery sectioning

Introduction

A variety of methods have been developed to study the physiopathology and treatment of stroke, and present knowledge of the molecular mechanisms that result in damage during and after brain ischemia (Small *et al*., 1999; Onteniente *et al*., 2003) is mainly based on detailed studies in animal models. The information gained from animal experimentation opened the possibility of pharmacological manipulation to reduce death and limit tissue damage and neuronal dysfunction. Such animal models have shown the existence of a large number of compounds with different mechanisms of action that reduce tissue damage after brain ischemia (Read *et al*., 1999). However, despite having been proven highly effective in reducing ischemic lesion size in animals, none of these compounds reduces mortality and/or attenuates disability in stroke patients (De Keyser *et al*., 1999; Cheng *et al*., 2004). Therefore, the value of these models to predict the usefulness of chemicals in humans with stroke has been questioned (Wiebers *et al*., 1990; Hunter *et al*., 1995; Green *et al*., 2003). Discrepancies between results from animal models of stroke and from clinical studies substantiate the continuous efforts to develop alternative methods and strategies to identify clinically effective drugs.

Previous studies from this laboratory showed that sequential common carotid artery sectioning (SCAS) produces a reproducible pattern of mortality, extensive brain damage (cerebral cortex, striatum, and hippocampus), and a wide range of neurobehavioral alterations in mice (Rodriguez *et al*., 2000; 2005). This new model of experimental ischemic stroke is essentially a model of global ischemia but it incorporates the idea of provoking an acute ischemic insult in aged animals subjected to chronic hypoperfusion, and, unlike most

*Author for correspondence; E-mail: rodcar@servidor.unam.mx

traditional models of ischemia, uses neurological and survival end points to determine the effects of brain ischemia and the degree of neuroprotection (Rodriguez *et al*., 2003). Our strategy to produce brain ischemia and to evaluate functional outcome and neuroprotection is technically simple. However, it is laborious and time consuming, and requires a large number of animals to demonstrate valid neuroprotection. As SCAS is not totally adequate for primary screening, we decided to search for a more practical and economic way to screen potential neuroprotective compounds.

There is much evidence to indicate that the guinea-pig ileum could be a useful model system for studying neuronal ischemia and neuroprotection. Many properties of the enteric nervous system (ENS) resemble those of the central nervous system (CNS) (Wood, 1994; Gershon, 1999). The ENS is a collection of neurons secreted into the myenteric and submucosal plexuses (Lomax & Furness, 2000) that can function relatively independently from the CNS and control or modulate motility, exocrine and endocrine secretions, microcirculation, and immune and inflammatory processes (Hansen, 2003a). It produces 25 or more potential transmitter substances, including peptides, amines, amino acids, and the monoxides, nitric oxide, and carbon monoxide (Costa *et al*., 1996; Goyal & Hirano, 1996; Furness *et al*., 1999), any of which might be involved in the transmission at enteric synapses. As a result of these characteristics, the guinea-pig isolated ileum and the myenteric plexus-longitudinal muscle preparations (MPLM) have been model systems widely used in pharmacology (Brookes, 2001; Hansen, 2003b). Like all other organs, survival and integrity of the small intestine requires a continuous supply of oxygen and glucose, and ischemia/reperfusion of the gut apparently triggers a cascade of pathological molecular events that lead to disturbed calcium homeostasis, excessive release of excitatory amino acids, and generation of nitrogen and oxygen free radicals (Kimball & Mulholland, 1995; Kirchgessner *et al*., 1997; Liu *et al*., 1997; Kong *et al*., 1998), which presumably play a central role in the pathogenesis of stroke-associated neuronal injury in the CNS (Peruche & Krieglstein, 1993; Small *et al*., 1999; Del Zoppo *et al*., 2000).

Today, much emphasis has been placed on the effect of intestinal ischemia/reperfusion on mucosa (Kong *et al*., 1998; Mallick *et al*., 2004), and only few experimental studies have dealt with their effect on enteric neurons and on intestine contractile function, even though such studies are crucial in the understanding of the neuronal death/survival mechanisms involved in human intestinal stroke. To our knowledge, the guinea-pig ileum has never been used to study neuroprotection. The present study evaluates effects of progressive ischemia *in situ* on contractile responses of the guinea-pig isolated ileum and MPLM preparations to electrical stimulation and to four chemicals that act on the smooth muscle through different mechanisms, and it explores the potential use of this preparation to study neuroprotection. Structural changes produced by intestinal ischemia were also examined.

Methods

Animals

Studies were performed in adult male guinea-pigs, weighing 600–900 g, obtained from our breeding facilities. Animals were housed one per cage in a temperature-controlled room ($22 \pm 2^{\circ}C$) with an automatically timed cycle of 12 h light/dark (lights on 0800–2000 hours). Food (Purina Chow, St Louis, MO, U.S.A.) and water were available *ad libitum*. At 24 h before experiments, food was withheld and free access to water was maintained. Surgery and experiments were performed between 0700–1400 hours. This study was carried out in accordance with the Declaration of Helsinki, and in compliance with regulations formulated by the National Health Ministry (Mexico).

Intestinal ischemia in situ

Guinea-pigs were anesthetized with pentobarbital ($142\,\mu mol\,kg^{-1}$, i.p.) and the terminal portion (25–30 cm) of the ileum was exposed by a midline incision. The mesenteric arteries were identified and delineated. Without considering the 10 cm nearest to the cecum, blood supply to a selected portion of the ileum (approximately 15 cm) was interrupted by clamping the corresponding ileal branches of the superior mesenteric artery. Few minutes after clamping, the intestinal segment changed color and became dark red, indicating successful ischemia, and clearly demarcated from the intestine with normal blood flow. As clamping of ileal branches of the mesenteric artery is not sufficient to completely block blood flow between adjacent intestinal segments (Yano *et al*., 1997; Lindestrom & Ekblad, 2004), we also sectioned the gut on each side of the intestinal loop selected for ischemia. The exposed intestine was thoroughly wetted with a cotton swab soaked in Krebs-bicarbonate (K-B) solution and covered during the time of ischemia. After logarithmically spaced periods of ischemia had elapsed (5, 10, 20, 40, 80, and 160 min), ischemic and nonischemic portions were removed and placed in a Petri dish with K-B maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. The K-B contained (mM) the following: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11; and choline chloride, 0.3. Intraluminal contents were flushed out with K-B solution.

Tissue preparation

In one group of experiments, 2.0-cm long portions of the ileum (intact ileum), three ischemic and one nonischemic, from the same guinea-pig, were cut and mounted in a 30 ml organ-bath containing K-B solution maintained at 37°C and constantly bubbled with a mixture of O_2 and CO_2. The upper end of the ileum was attached to a Grass FT-03C force displacement transducer connected to a Grass 7B polygraph to record isometric contractions. The resting tension was fixed at 1 g.

In the second set of experiments, the MPLM was dissected using the method described by Ambache (1954). Briefly, segments of ileum of about 3 cm long, three ischemic and one nonischemic, obtained from the same animal, were stretched onto a glass rod of 6 mm diameter, and the longitudinal muscle with its myenteric plexus was separated from the rest of the intestine by gentle circular strokes with a wisp of cotton soaked in K-B solution applied along the mesenteric attachment. Each strip of tissue (about 25 mg) was then suspended in a 30 ml organ bath containing K-B solution at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. The strips were suspended under the resting tension of 1 g.

In the last group of experiments, ilea were taken from animals not subjected to intestinal ischemia, and intact segments and MPLM strips were prepared and stimulated as described above. This group served as control.

Chemical and electrical stimulation

The preparations were allowed to equilibrate for 60 min under continuous superfusion (10 ml/min) of warm K-B solution, continuously bubbled with 95% O_2 and 5% CO_2 to maintain the pH at 7.4, and then stimulated with acetylcholine (ACh) (1×10^{-9}–1×10^{-4} M) to ascertain their suitability. Thereafter, intact preparations were subjected to electrical field stimulation through two nickel electrodes that were positioned parallel to the segments. The anode was placed intraluminally. The MPLM preparations were stimulated through two electrodes placed at the top and bottom of the organ bath and separated by 3 cm. In both preparations, rectangular current pulses of 3.0 ms duration and of sufficient voltage (progressively increased up to 40 V) to produce maximal responses were applied to the tissue at 0.3 Hz by means of a Grass S88 stimulator. As preliminary studies indicated that electrically induced contractions in nonischemic and ischemic segments do not change over a period of 3 h of continuous electrical stimulation, this was maintained for periods of only 5–10 min. Superfusion was not suspended during electrical stimulation, and at the end of this period, the same preparations were used to retest the contractile response to ACh and to evaluate the effect of other smooth muscle stimulants. Noncumulative concentration–response curves were constructed for ACh (1×10^{-9}–1×10^{-4} M), histamine (HIS, 1×10^{-9}–1×10^{-4} M), potassium chloride (KCl, 1×10^{-3}–3.2×10^{-2} M), and barium chloride ($BaCl_2$, 1×10^{-5}–1×10^{-2} M).

Drugs were dissolved in saline and added to the bath in a volume of 0.3 ml. In all cases, after the maximal contractile effect had been obtained (10 s), the preparation was washed with 30–60 ml of warm K-B solution, and the chamber was continuously superfused between drug administrations. Concentrations are expressed as final drug concentrations actually in contact with strips, and cover the full range from no effect to maximal contractile response.

Electron microscopy

At the end of the experimental period, ischemic and nonischemic segments were removed from the organ baths and immersed in a fixative solution containing 2% paraformaldehyde and 2.0% glutaraldehyde in 0.1 M sodium phosphate-buffered saline, pH 7.4. Segments were removed after 2 h and cut in small blocks (1 mm^3), washed with cacodylate buffer, postfixed in 1% osmium tetroxide for 2 h, dehydrated in an ethanol series, and embedded in Eppon 812. Semithin sections (60–80 nm thick) were contrasted with uranyl acetate and Reynold lead citrate. Sections were observed and photographed with a Zeiss EM-10 electron microscope.

Statistical analyses

In all cases, the contractile responses were expressed as grams of tension, and concentration–response data were analyzed for maximal response (E_{max}). Results were expressed as the mean values ± s.e.m. for a number of experiments, n, as indicated in the figure legends. Multiple comparisons against control preparations were made using one-way ANOVA, followed by Dunnett's test. A value of $P<0.05$ was considered as significant. Analysis was performed using the GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA, U.S.A.).

Drugs

ACh and HIS were purchased from Sigma (ST Louis, MO, U.S.A.); KCl and $BaCl_2$ were bought from J.T. Baker (Phillipsburg, NJ, U.S.A.). All drugs were dissolved in saline solution (0.9%).

Results

Spontaneous activity

Ischemia *in situ* consistently decreased the size of spontaneous contractions registered *in vitro*. The spontaneous contractions in ischemic segments ($\geqslant$20 min) were generally <0.3 g in intact segments, and <0.1 g in MPLM preparations, whereas nonischemic segments usually displayed spontaneous activity >0.5 and 0.2 g, respectively. In both preparations, differences in size of spontaneous contractions between ischemic and nonischemic segments were statistically significant at 40, 80, and 160 min of ischemia.

Responses to electrical stimulation

Nonischemic intact segments started responding to $\leqslant$8 V electrical stimuli; and maximal electrical stimulation (40 V) typically evoked a contraction normally consisting of two components: a phasic response, characterized by a sharp spike lasting few seconds, followed by a small, tonic, slowly declining response. In contrast, ischemic segments ($\geqslant$20 min) required $\geqslant$12 V to respond, and maximal stimulation did not elicit the small, secondary contraction. Nonischemic and ischemic MPLM preparations started to respond to electrical stimulation at $\geqslant$12 V and $\geqslant$16 V, respectively, and maximal stimulation (40 V) produced only single twitches. It should be noted that mean response of nonischemic MPLM preparations (1.1 ± 0.1 g) to electrical stimulation was substantially lower than that of nonischemic intact segments (2.4 ± 0.3 g of tension).

Short periods of ischemia (5–20 min) only slightly decreased the reactivity of intact segments to electrical stimulation, whereas a 40, 80, and 160 min ischemia resulted in an approximately 56, 64, and 76% decrease in the contractile response of the ileum to electrical stimulation (Figure 1). In contrast, significant reductions in size of responses of MPLM preparations to electrical stimulation were observed as early as 5 min of ischemia, and contractions progressively decreased and were almost completely abolished in strips subjected to 160 min of ischemia.

Responses to chemical stimulation

The two types of ischemic strips, intact segments, and MPLM preparations were contracted by the four smooth muscle

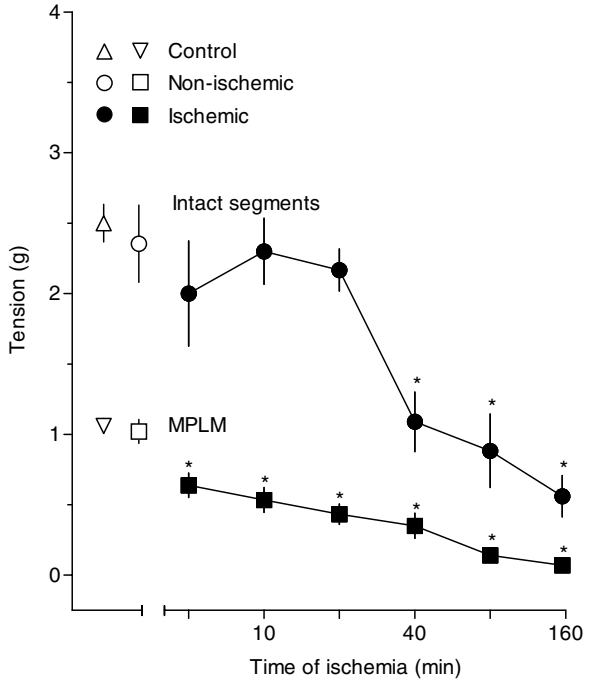

Figure 1 Effect of various times (5–160 min) of intestinal ischemia *in situ* on the responses of intact segments and myenteric plexus-longitudinal muscle preparations to electrical stimulation (40 V, 0.3 Hz, 3.0 ms). Contractions are expressed as grams of tension. Each point is the mean ± s.e.m. for 6–9 individual preparations. *Significantly different from control (one-way ANOVA, followed by Dunnett's test).

stimulants tested, and all produced a concentration-dependent contraction (data not shown). Both types of strips were more sensitive to ACh and HIS, 1×10^{-9} M being the threshold concentration, and were clearly less sensitive to KCl (1×10^{-3} M) and $BaCl_2$ (1×10^{-5} M). In ischemic and nonischemic MPLM preparations, size of contractions induced by stimulants was consistently lower than those of the intact segments. For example, the response obtained with the highest concentration of ACh (2.6 ± 0.2 g) was less than 42% of the maximum response in the intact segments (4.5 ± 0.2 g).

Figure 2 compares the mean E_{max} values for stimulants in nonischemic and ischemic intact segments after various times of ischemia. The E_{max} values in intact strips subjected to short periods of ischemia (5–20 min) were about the same or consistently greater than those of nonischemic strips. In contrast, E_{max} values for both receptor and nonreceptor mediated stimulants were significantly reduced ($P < 0.05$) in segments subjected to large periods of ischemia (40–160 min). In MPLM preparations, 5 min of ischemia did not modify E_{max} values (Figure 3), but maximal responses to stimulants tended to be reduced after larger periods of ischemia.

Finally, and as shown in Figures 1–3, there were not significant differences between control (ilea taken from animals not subjected to intestinal ischemia) and nonischemic preparations.

Ultrastructure

Electronic microscopy revealed no changes in neurons or in smooth muscle cells of nonischemic strips (Figure 4a). Short and intermediate periods of ischemia (5–40 min) produced no significant changes in neurons or in smooth muscle cells. Condensation of chromatin, constriction of nucleus, partial bulging of the outer nuclear membrane, destruction of organelles, and focal necrosis with fusion of the plasmalemma and part of the sarcoplasm were seen in strips subjected to 80–160 min of ischemia (Figure 4b).

Discussion

Stroke occurs when cerebral blood flow severely decreases (< 10 ml/100 g/min) or is totally interrupted. Oxygen and energy deficits due to cessation or reduction of blood flow trigger a cascade of biochemical perturbations that eventually result in death of neurons, glia and blood vessels unless blood flow is promptly restored. In humans and laboratory animals, neuronal damage following cerebral ischemia can produce death, and has a debilitating effect on survivors, with symptoms ranging from motor incoordination and paralysis to impairments in memory and cognition (Baumlin & Richardson, 1997; DeVries *et al*., 2001). Our results show that interruption of blood supply *in situ* with superfusion *in vitro* significantly affects the motor function of the intestine, and produces damage and cell death of the myenteric neurons. We found that intestinal ischemia consistently reduced the size of spontaneous contractions, increased the threshold to electrical stimulation, and resulted in time-dependent alterations in the responsiveness of the guinea-pig isolated ileum, to both electrical and chemical stimulation.

The most important finding of the present study showed that contractile responses of ischemic tissues to neurogenic stimulation decreases earlier and to a significantly greater extent than did non-nerve mediated responses of smooth muscle**.** Electrical stimulation of intact segments and MPLM preparations releases various neurotransmitters, mainly ACh (Bornstein *et al*., 2004), from functional synapses. These transmitters subsequently activate postsynaptic receptors on the surface of smooth muscle cells. Stimulation of specific receptors elicits contraction *via* activation of G proteins that are coupled to a diverse set of downstream signaling pathways and effector proteins. These events lead to elevated intracellular calcium concentration, which activates myosin light chain kinase to phosphorylate and activate myosin II, thus, causing contraction (Gerthoffer, 2005). Therefore, the decline of the electrically evoked contractions seen in this study could be due to either a reduced transmitter release, or to a reduction in the ability of the smooth muscle to generate force in response to the released neurotransmitters. As can be seen in Figures 2 and 3, the contractile responses of ischemic segments to ACh and HIS, which act directly on specific smooth muscle receptors; KCl, which depolarizes the plasma membrane, resulting in activation of voltage-dependent Ca^{2+} channels followed by extracellular Ca^{2+}-dependent contraction (Bolton, 1979); and $BaCl_2$, which enters the intracellular space and directly stimulates the contractile mechanism (Hansen *et al*., 1984), were considerably less affected than the responses to electrical stimulation. This suggests that the decline in response to electrical stimulation is due to changes in transmitter release from the nerve terminals, and indicates that ischemia/superfusion (I/S) damages first, and more severely, enteric neurons than specific receptors, intracellular

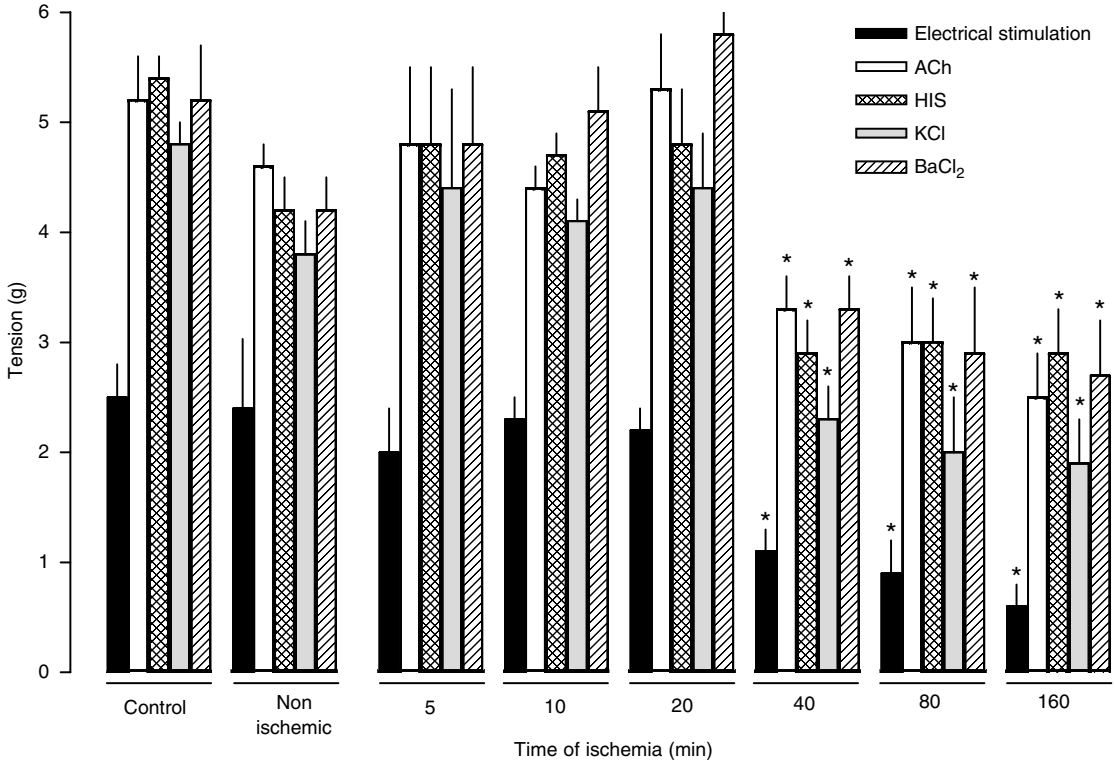

Figure 2 Effect of various times (5–160 min) of intestinal ischemia *in situ* on the responses *in vitro* of intact segments to electrical stimulation and to stimulants that act on the smooth muscle through different mechanisms. Preparations were allowed to equilibrate for 60 min under continuous superfusion of K-B solution and then electrically stimulated at 40 V (0.3 Hz, 3.0 ms). Thereafter, complete noncumulative concentration–response curves were obtained for acetylcholine (ACh), histamine (HIS), potassium chloride (KCl), and barium chloride ($BaCl_2$). In each case, maximum response achieved is expressed in grams of tension. Each bar is the mean $\pm$ s.e.m. for 6–9 individual preparations. *Significantly different from control (one-way ANOVA followed by Dunnett's test).

signal transduction pathways, and/or the contractile apparatus of the smooth muscle. Apparently, the inhibitory effects of I/S are more functional than structural. By electron microscopy, we found that ischemic insults of 5, 10, 20, and 40 min did not produce significant degrees of structural damage. We postulate that the decline in responses to electrical stimuli is a major indicator of neuronal functional injury after I/S. As the reduced responses to neurogenic stimulation can be quantitated, it is likely that this parameter can be an accurate measure for assessing different treatment modalities aimed at the reduction of neuronal ischemic injury.

We took advantage of this finding and, in a complementary group of experiments, we added dexrazoxane (1×10^{-4} M) to the K-S solution reservoir, and ischemic and nonischemic strips were perfused for 2 h before stimulation. Dexrazoxane did not modify the amplitude of responses to chemicals, but significantly ameliorated the decrease in size of electrically evoked responses in ischemic, intact, and MPLM preparations (data not shown). Dexrazoxane is a powerful synthetic iron chelator that has been successfully used to reduce cardiac toxicity in patients receiving anthracycline-based chemotherapy for cancer (Seifert *et al*., 1994). Dexrazoxane has also been shown to reduce neurological alterations and mortality in mice with severe forebrain ischemia (Rodriguez *et al*., 2003). Its protective activity against ischemia seems to involve iron-dependent free radical reactions, that is, preventing iron catalyzed formation of $^{\bullet}$OH. The fact that dexrazoxane ameliorates the decrease in the maximal response to electrical stimulation is in line with the idea that the guinea-pig ileum could be a useful model system to study neuroprotection. However, a more complete pharmacological characterization is required.

Much research prior to this study focused on the effects of ischemia on intestinal mucosa (Kong *et al*., 1998; Mallick *et al*., 2004), but few experimental studies have dealt with the effects of ischemia on enteric neurons and on intestine contractile activity. Decreased intestinal transit (Udassin *et al*., 1995; Hassoun *et al*., 2001), dysfunction of piglet migrating motor complex (Hebra *et al*., 1993), as well as decreased ileum contractility in response to bethanecol, ACh, KCl, and electrical stimulation (Hierholzer *et al*., 1999; Ballabeni *et al*., 2002; Ozacmak *et al*., 2005) have been described as consequences of ischemia/reperfusion produced in various experimental models. Yano *et al*. (1997) reported decreased size in myenteric neurons in the jejunum after 2–6 h of ischemia followed by 2 days of reperfusion. Piao *et al*. (1999) observed that after 4-h of ischemia many myenteric neurons in rat small intestine degenerated within 4 weeks, and suggested that intestinal ischemia causes delayed neuronal cell death. Lindestrom & Ekblad (2004) found that ischemia (60 min) followed by reperfusion (1 h–10 weeks) reduces the total number of enteric neurons, and that the myenteric neuronal loss is not obvious until after the first week of reperfusion. Our results, indicating that ischemia *in situ*

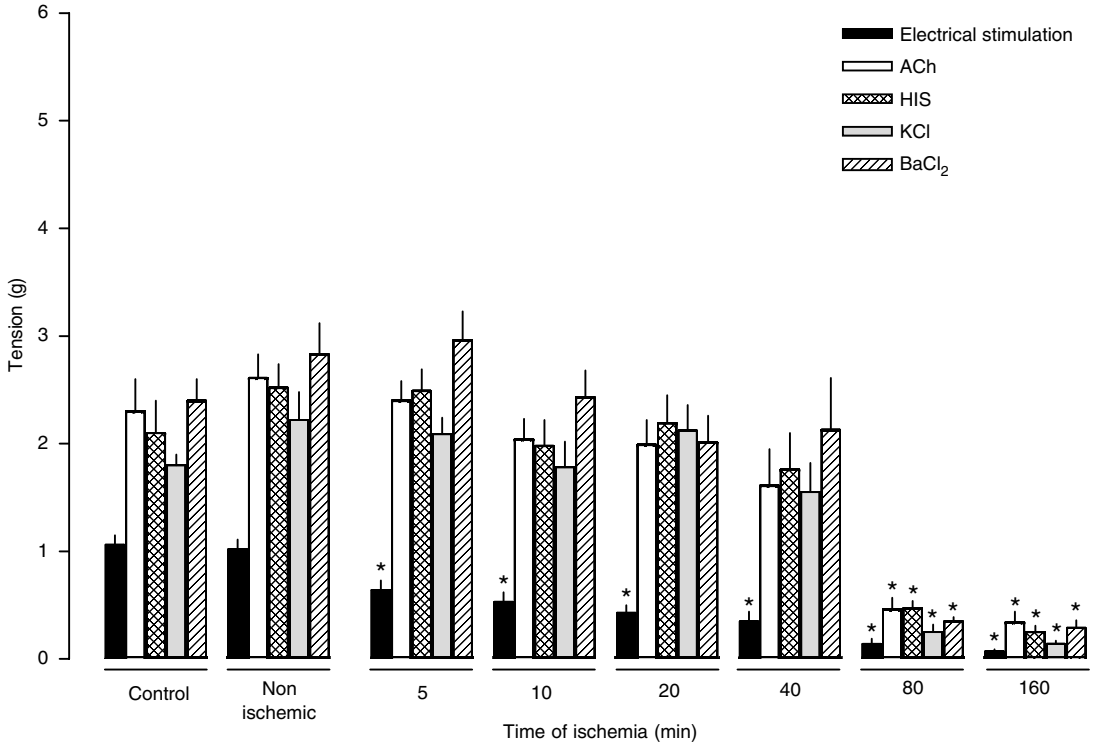

Figure 3 Effect of various times (5–160 min) of intestinal ischemia *in situ* on the responses *in vitro* of MPLM preparations to electrical stimulation and to stimulants that act on the smooth muscle through different mechanisms. Preparations were allowed to equilibrate for 60 min under continuous superfusion of K-B solution and then electrically stimulated at 40 V (0.3 Hz, 3.0 ms). Thereafter, complete noncumulative concentration–response curves were obtained for acetylcholine (ACh), histamine (HIS), potassium chloride (KCl), and barium chloride ($BaCl_2$). In each case, maximum response achieved is expressed in grams of tension. Each bar is the mean ± s.e.m. for 6–9 individual preparations. *Significantly different from control (one-way ANOVA followed by Dunnett's test).

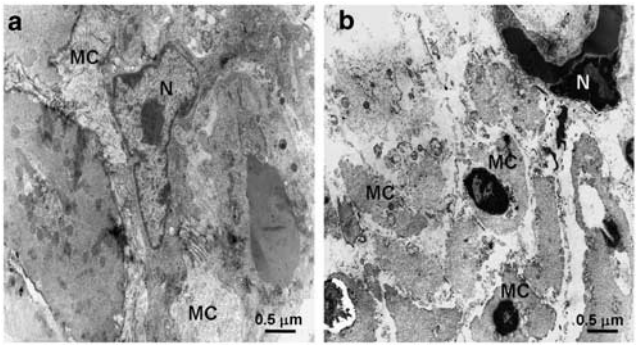

Figure 4 (a) Electron micrographs of guinea-pig nonischemic intestinal muscular cells (MC) and myenteric neurons (N). (b) Myofibrilar lysis and picnotic nuclei in muscular cells (MC) and necrotic neurons (N) are evident after 160 min of intestinal ischemia.

followed by superfusion *in vitro* alters the responsiveness of the intestine to electrical and chemical stimulation, and causes structural damage in the ENS, are consistent with these previously published data using ischemia/reperfusion models.

When the results from intact and MPLM preparations were compared, two important differences emerged: (a) the maximal contractile responses to electrical and chemical stimulation were significantly less in MPLM than in whole ileum preparations. The simplest explanation for this difference is related to the muscle mass of preparations under study (Moreels *et al*., 2001). It is also possible that in preparing MPLM there was some damage either to the myenteric plexus or to the longitudinal smooth muscle making it a less sensitive system than the whole ileum preparation; (b) short periods of ischemia (5–20 min) consistently increased the contractile response to smooth muscle stimulants in intact segments but not in MPLM preparations. This difference can be attributed to the presence of all the intestinal wall components in intact segments. Recently, it has become apparent that intestinal ischemia causes an acute inflammatory response that is particularly enhanced by reperfusion (Hierholzer *et al*., 1999; Stojadinovic *et al*., 1999; Takahashi *et al*., 2001; Stallion *et al*., 2002). Thus, it is possible that the increased responsiveness, seen after short periods of ischemia, is related to an acute inflammatory process and/or the presence of inflammatory mediators. In support of this point of view is the fact that the increased responsiveness, seen in intact segments, tends to be rather unspecific; it develops in response to all four stimulants at roughly the same degree, irrespective of the type of chemical stimulus. It is unclear why short periods of ischemia did not also increase the response to electrical stimulation. The best explanation could be that the increased reactivity was masked by enteric neuron damage. This possibility emerges from the different pattern of decreased contractility between the whole ileum and MPLM preparations (Figures 2 and 3).

In conclusion, results show that contractile responses to neurogenic stimulation decreased earlier and to a significantly greater extent than the non-nerve mediated responses of the ischemic intestinal smooth muscle. We propose that reductions of maximal responses to electrical stimulation can be used to study neuroprotection. However, a complete pharmacological characterization of this model is needed to validate its capacity to detect useful neuroprotective activity. Additional studies to determine the minimum time required, after superior mesenteric artery occlusion, for structural abnormalities to become discernible are currently being carried out.

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