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Reduced capacitative calcium entry in the mesenteric vascular bed of bile duct-ligated rats

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Abstract

In this work, we analyzed the interaction of nitric oxide (NO) with some of the mechanisms known to regulate intracellular calcium levels in order to gain insight into the mechanisms responsible for the reduced vascular pressor response to vasoconstrictors observed in an experimental model of liver cirrhosis. Specifically, we hypothesized that the entry of calcium through capacitative channels is defective in this model. The experiments were performed with isolated, Krebs-perfused and de-endothelialized mesenteric arterial bed of rats with bile duct ligation (4 weeks) and their controls. Pretreatment with thapsigargin to inhibit calcium uptake into sarcoplasmic reticulum potentiated the pressor responses to methoxamine, but the response of the cirrhotic vessels was significantly lower than that of the controls. Under the same conditions, perfusion of the mesenteries with zero calcium-Krebs resulted in lower pressor responses to methoxamine, especially in the mesenteries of the bile duct-ligated rats. To specifically analyze the entry of calcium through store-operated calcium channels, the pressor response to the addition of calcium was studied in mesenteries perfused with zero calcium-Krebs and in the presence of thapsigargin. Again, the response of the cirrhotic mesenteric beds was significantly lower than that of the control vessels. Under all these experimental conditions, the differences between control and cirrhotic responses were abolished by pretreatment with the NO synthesis inhibitor N^{w}-nitro-L-arginine (NNA). These results indicate that, in the mesenteric bed of bile duct-ligated rats, an excess of nitric oxide interferes with the release of calcium from thapsigargin-sensitive internal stores and also reduces the capacitative entry of calcium into vascular muscular cells induced by the depletion of calcium from internal stores. This mechanism may have an important role in the reduced pressor response observed in the mesenteric vascular bed in cirrhosis.
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1. Introduction

Calcium is an important regulator of vascular muscle contraction. The vascular smooth muscle cells use calcium as the trigger for contraction. A number of vasoconstrictor and vasodilator hormones and factors act to increase or decrease, respectively, intracellular calcium levels and, therefore, modulate the activity of the contractile apparatus of muscle cells and hence the diameter and resistance of the blood vessels (Himpens et al., 1995; Karaki et al., 1997).

One of the most important features of liver cirrhosis is the splanchnic and systemic arterial vasodilatation, related to both an increase in vascular capacity and an active vasodilatation. This arterial vasodilatation seems to be the consequence of the excessive generation of vasodilating substances, which also contributes to a lower than normal pressor response to circulating nervous or humoral substances (Blendis and Wong, 2001). Previous studies from our and other laboratories have shown that nitric oxide (NO) is an important contributor to this well-known phenomenon of vascular hyporesponsiveness to vasoconstrictors observed in experimental models of liver cirrhosis and portal hypertension (Sieber and Groszmann, 1992; Ortíz et al., 1996; Atucha et al., 1996a,b, 1998, 2000; Nadal et al., 2002). Specifically in the arterial mesenteric bed of portal hypertensive and cirrhotic ascitic rats, excess nitric oxide

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reduces the agonist-induced vascular contraction, mostly through the formation of cGMP (Atucha et al., 1998, 2000). It has also been observed that NO interferes with some of the mechanisms that regulate the level of intracellular calcium, both in the mesenteric vascular bed and in isolated smooth muscle cells from cirrhotic rats (Nadal et al., 2002; Atucha et al., 2003). In a previous study of cirrhotic rats, we (Nadal et al., 2002) observed that NO reduced calcium entry from the extracellular space through both receptor and voltage-operated calcium channels and also found evidence of altered entry of calcium through capacitative channels, that is the entry of calcium induced by the depletion of internal stores. Thus, in order to prove the hypothesis of an altered entry of calcium through capacitative channels in the mesenteric bed of bile duct-ligated animals, we analyzed specifically the interaction of NO with some of the mechanisms that control the release of calcium from the internal stores and the associated entry of calcium through store-operated channels.

2. Methods

Male Sprague–Dawley rats born and raised in the Animal House of the Universidad de Murcia were used in the present study. All the experiments were performed according to the rules for the treatment of laboratory animals of the European Union.

2.1. Experimental groups

Animals weighing around 200 g were subjected to bile duct ligation and excision or sham operation (control) as previously described (Ortíz et al., 1996; Nadal et al., 2002; Atucha et al., 2003). All the animals were used in the fourth week after bile duct ligation (23–25 days).

2.2. Isolation and perfusion of the mesenteric bed

This technique was performed as previously described (Atucha et al., 1996a,b, 1998, 2000, 2003; Nadal et al., 2002). Briefly, the superior mesenteric artery was cannulated using a PE-60 catheter and gently perfused with 15 ml of warmed Krebs solution to eliminate blood. After the superior mesenteric artery was isolated with its mesentery, the gut was cut off near its mesenteric border. The mesenteric bed was then placed in a 37 °C water-jacketed container and perfused at a constant rate (4 ml/min) with oxygenated 37 °C Krebs solution (95% O_2, 5% CO_2) using a roller pump (Masterflex 7523-35, Cole-Parmer Co., Barrington, IL). The Krebs solution had the following composition (mM): NaCl, 118; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; EDTA, 0.026; and glucose, 11.0; pH 7.4. The preparation was covered with a piece of Parafilm (American National Can, Greenwich, CT) to prevent drying. Perfusion pressure was measured with a transducer (Hewlett-Packard 1280) on a side-arm just before the perfusing cannula and continuously recorded on a polygraph inscriber (Hewlett-Packard 8805D). Since flow rate was kept constant throughout the experiment, pressure changes reflect vascular resistance changes. The preparation was allowed to recover for at least 30 min and then the experimental protocol was performed. Perfusion pressure at each concentration was allowed to plateau before the addition of the next higher concentration. Only one concentration–response curve was performed with each preparation. All the experiments were performed with mesenteric beds in which the vascular endothelium had been removed by a brief treatment with cholic acid in distilled water, as described previously (Atucha et al., 1996a,b). The validity of this procedure was checked at the end of the experiment, by administering acetylcholine (10 μM) to the constricted mesenteric bed. A response less than 10% of the control response (usually 90% relaxation) was considered to indicate the mesenteric bed was de-endothelialized.

2.3. Experimental protocols

On the day of the experiment, the animals were anesthetized (Inactin, 100 mg/kg, i.p., RBI, Natick, MA, USA) and the mesenteric bed was isolated and perfused as described above. The following protocols were performed:

2.3.1. Response to methoxamine in thapsigargin-pretreated mesenteric vascular beds

A cumulative dose–response curve for methoxamine (1–1000 μM) was carried out in the presence of thapsigargin (3 μM), to block the calcium ATP-ase of the sarcoplasmic reticulum. These experiments were performed in the absence and in the presence of N^{w}-nitro-L-arginine (NNA, 100 μM, 5 animals per group and condition). The pretreatment period was 30 min, the time necessary for the preparation to stabilize at a new perfusion pressure. Then, thapsigargin and NNA were present throughout the experiment.

2.3.2. To analyze solely the intracellular release of calcium

The vessels were perfused with zero-calcium Krebs (nominally calcium-free, with no EGTA added) containing thapsigargin (3 μM). After stabilization, a dose–response curve for methoxamine was performed, both in the absence and in the presence of NNA (5 animals per group and condition). In a further two groups (3 animals each), the experiment was performed similarly but also including methoxamine (7 μM) during the pretreatment to completely deplete the internal stores.

2.3.3. To analyze the role of calcium entry through capacitative calcium channels

The vessels were perfused with a zero-calcium and thapsigargin-containing (3 μM) buffer. After stabilization, the response to the addition of calcium (0.01–1 mM) was examined both in the absence and in the presence of NNA (5 animals per group and condition).

2.4. Drugs

All the products used were from Sigma Chemical (Madrid, Spain). Drug stock solutions were prepared in distilled water

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