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Structure-related blockage of calcium channels by vasodilator alkamides in mice mesenteric artery

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ABSTRACT

The development of new calcium channel blockers is still relevant for the understanding of their physiological role and pharmacological and therapeutic purposes. For this task, natural products represent a relevant source of new drugs. The present work investigated the mechanism and the structural relationship of the vasodilator effect of riparins I, II and III in mouse small mesenteric artery. Riparins I, II and III induced an endothelium-independent and concentration-dependent vasodilator effect in mesenteric arteries. Riparins II and III were more potent than riparin I, suggesting a structural relationship of the effect of these drugs. All riparins inhibited the contractile effect of KCl, similarly to nifedipine. However, the inhibitory profile was different for the contractile responses to phenylephrine and caffeine, passing from similar to nifedipine with riparin I, for similar to SKF-96365 with riparin III. A comparable effect was observed for the increase in the intracellular calcium concentration induced by caffeine and phenylephrine. These results suggest that the higher hydroxylation provides the alkamides the ability to inhibit non-selective cation channels in addition to the inhibition of L-type calcium channels in mouse mesenteric arteries. These observations may give support to the development of new selective inhibitors of non-selective cation channels using alkamides as leading compounds.

1. Introduction

The regulation of small arteries tonus is essential for the control of the blood pressure. The processes underlying smooth muscle contraction can involve the activation of voltage and receptor operated Ca^{2+} influx and the mobilization of Ca^{2+} from the intracellular stores [1]. The influx of Ca^{2+} plays a pivotal role in the initiation and maintenance of the tonus of resistance arteries [2]. The voltage-gated L-type Ca^{2+} channels (LTCCs) represent the principal structure managing the influx of Ca^{2+} in vascular smooth muscle cells (VSMCs) from resistance arteries [3]. However, the influx of Ca^{2+} also occurs by non-selective cation channels located in the plasmatic membrane. The superfamily of transient receptor potential (TRP) channels is permeable to Ca^{2+} and can be activated by a wide variety of stimuli [4]. In this sense, the canonical TRP (TRPC) channels are essential in the vascular smooth muscle. The TRPC1, for example, is described as a store-operated calcium channel (SOC) involved in the refilling of intracellular Ca^{2+} stores [5] and as a mechanosensitive stretch-activated cation channel (SAC) [6]. The TRPC3 and TRPC6 channels act as receptor-operated calcium channel (ROC) [7]. In addition to their physiological role, TRPC channels have been associated with pathophysiological conditions such as essential hypertension, pulmonary hypertension and age-related vascular cognitive impairment [8–10]. In the therapeutics of cardiovascular diseases, the inhibition of the Ca^{2+} influx is only represented by LTCCs blockers. The recent report on a new inhibitor of TRPC6 [11] demonstrates that the development of new drugs interfering with the Ca^{2+} signaling for the treatment of cardiovascular diseases is still an open field.

Natural products are a constant inspiration for the development of new drugs. Consequently, the investigation of drugs with smooth muscle relaxant activity can represent a good starting point. Alkamides (Fig. 1) such as riparin I (N-benzoyltyramine), riparin II (N-(2-hydroxybenzoyl)-tyramine) and riparin III (N-(2,6-dihydroxybenzoyl)-tyramine) are smooth muscle relaxant drugs isolated from the fruits of *Aniba riparia* [12,13]. These preliminary reports described riparins as inhibitors of LTCCs and riparin III was significantly more potent than the other two. However, these studies were based on indirect evidence. Additionally,

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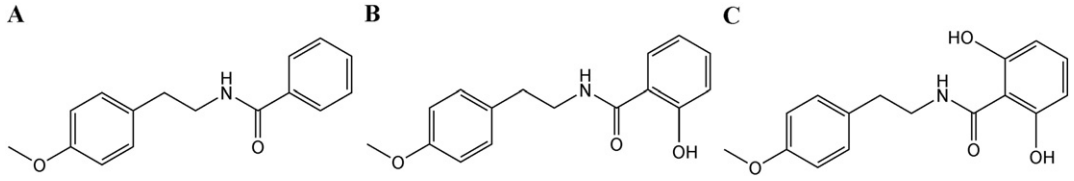

Fig. 1. Molecular structure of riparins I (A), II (B) and III (C).

riparins were described as antinociceptive, anti-depressant, anxiolytic and anti-inflammatory drugs [14–17].

Considering the importance of the resistance arteries for the control of the blood pressure and the predominant participation of the Ca^{2+} influx for the contraction of resistance arteries [18], the present work investigated the underlying mechanism of the vasodilator effect of riparins I, II and III in mice mesenteric artery and their structure–activity relationship. The present study shows, by functional and fluorescence microscopy experiments, that alkamides are vasodilator drugs and the increase in hydroxylation confers the ability to inhibit non-selective calcium channels in addition to the inhibition of LTCCs.

2. Materials and methods

2.1. Animals

The protocols were in accordance with the guidelines for the ethics committee [protocol No. 263/2013, Ethics Committee (CETEA) of the Universidade Federal de Minas Gerais (UFMG)]. Male Swiss mice (8–12 weeks old) were used, with approximately 35 to 40 g of body weight. All animals were obtained from the animal facility of the Institute of Biological Sciences — UFMG. All mice were maintained at five per cage at a constant temperature (23 °C), with a 12 h dark/light cycle. Free access to standard chow was allowed and filtered water was supplied *ad libitum*.

2.2. Small mesenteric artery preparation and mounting

Small mesenteric arteries were prepared as previously described [19]. Briefly, male Swiss mice were euthanized by decapitation, the viscera were exposed, and a proximal segment of the small bowel was removed and pinned in a dissecting dish containing physiological salt solution (PSS) of the following composition (in mmol/l): NaCl, 119; KCl, 4.7; KH_2PO_4, 0.4; $NaHCO_3$, 14.9; $MgSO_4$, 1.17; $CaCl_2$, 2.5; glucose, 5.5. Branch II or III resistance arteries were cleaned of fat and connective tissue, and a segment 1.6 to 2.0 mm in length was removed. In some experiments, the endothelial layer was removed immediately after dissection. The segment was then mounted on previously described myograph [20]. The presence of functional endothelium was assessed by the ability of acetylcholine (ACh; 10 μM) to induce more than 70% relaxation of vessels precontracted with phenylephrine. When necessary the endothelium was removed by the gentle friction of the lumen with a tungsten wire. The absence of a relaxation response to ACh was taken as evidence that the vessel segments were functionally devoid of endothelium.

2.3. Vasorelaxant activity of riparins I, II and III in precontracted vessels

The vasorelaxant activity of riparins was measured in vessels with or without a functional endothelium precontracted with phenylephrine (3 μM). Riparins I, II and III were added in increasing cumulative concentrations once the response to phenylephrine had stabilized. The mesenteric arteries without a functional endothelium were also precontracted with PSS containing 50 mM KCl (equimolar replacement of NaCl with KCl).

2.4. Measurement of the intracellular calcium by fluorescence microscopy in mesenteric arteries

The fluorescent dye Fluo 4-AM (Invitrogen) was used for measuring the intracellular calcium signal. The arteries were isolated and placed in HEPES-PSS (mM) solution: NaCl, 130; HEPES, 10; glucose, 6; KCl, 4; $NaHCO_3$, 4; $CaCl_2$, 1.8; $MgSO_4$, 1.2; KH_2PO_4, 1.18; EDTA, 0.03, pH 7.4, as previously described [21]. Arterial rings (1.6 to 2 mm length) were transfixed by a stainless steel wire (40 μM diameter). These rings were placed in a light protected chamber containing Fluo 4-AM (5 μM) plus pluronic acid (5 μM), diluted in HEPES-PSS, for 30 min. After incubation, the arterial ring was removed from the chamber and mounted on a mounting plate. The excess of fluorescent dye was removed by washing the rings with HEPES-PSS solution twice in intervals of 15 min. After recording the basal fluorescence, an increase in the intracellular calcium was stimulated with caffeine (10 mM), KCl (50 mM) or phenylephrine (10 μM). For the high [K^+] HEPES-PSS, there was an equimolar reduction in NaCl concentration to the increase in the KCl concentration. Then the mesenteric artery rings were pre-incubated for 30 min with riparins I (100 μM), II (30 μM), III (30 μM) or nifedipine (10 μM) to caffeine and KCl stimulation. For the phenylephrine stimulation, riparins I, II and III were pre-incubated with nifedipine (10 μM) and compared with the calcium signal induced by phenylephrine in the presence of nifedipine alone. F_0 was used as the basal fluorescence before stimulation and F as the final fluorescence after stimulation, obtaining (F/F_0). Images were acquired by a Zeiss

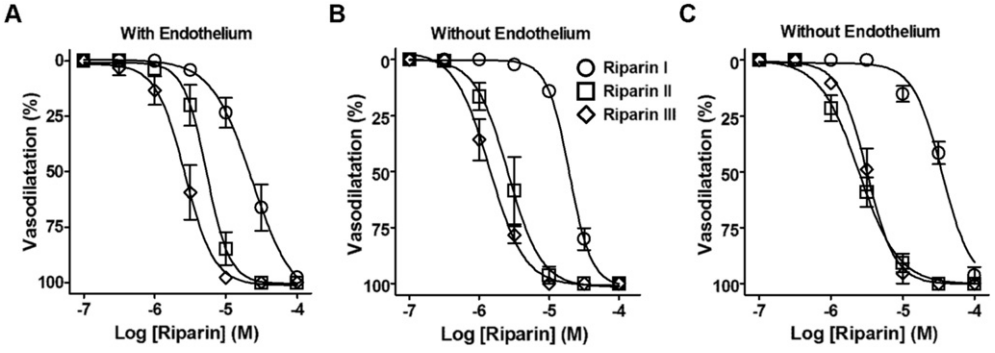

Fig. 2. Vasodilator effect of riparins I, II and III in the presence (A) and in the absence (B) of a functional endothelium in vessels precontracted with phenylephrine (3 μM) or KCl (50 mM; C). Results are the mean ± s.e. mean of five experiments.