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Short Communication

Mechanism of the vasodilator effect of Euxanthone in rat small mesenteric arteries

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ABSTRACT

In the present work we investigated the mechanism involved in the vasodilator effect induced by euxanthone in rat small mesenteric arteries. We observed that euxanthone induced concentration-dependent vasodilatation in arteries by a mechanism independent on the release of endothelial factors, such as nitric oxide (NO) and cyclooxygenase-derived factors. In addition our results also suggest that euxanthone induced its vasodilator effect through inhibition of calcium-sensitive mechanisms activated by protein kinase C, rather than by inhibition of contractions dependent on the release of the intracellular calcium stores or by inhibition of voltage-operated calcium channels.

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Introduction

Xanthones have been reported to mediate several important biological activities such as anti-inflammatory (Lin et al., 1996a, b, c), antithrombotic (Lin et al., 1996a, b, c) and anti-cancer (Lin et al., 1996a, b, c). Recent reports have shown that xanthones could either activate or inhibit different isoforms of protein kinase C (PKC) and, therefore, modulate an enormous quantity of biological effects (Saraiva et al., 2003; Saraiva et al., 2002a; Saraiva et al., 2002b). In the cardiovascular system, xanthones have been described as antihypertensive and vasodilator drugs (Capettini et al., 2009; Cheng and Kang, 1997; Wang et al., 2009), as well as protective for endothelial cells (Jiang et al., 2003). *Gentiana kokiana* is a xanthone-containing herb commonly used for the treatment of hypertension in Italy (Uncini-Manganelli et al., 2000). The vasodilator effect of crude extract and xanthones from *G. kokiana* was described as the mechanism of its antihypertensive activity (Baragatti et al., 2002; Chericoni et al., 2003).

Euxanthone (2,7-dihydroxyxanthone) (Fig. 1) was isolated from *Vismia latifolia* (Santos et al., 2000), a plant used in the popular medicine as tonic and febrifugal agent. This xanthone has been described as a modulator of PKC expression (Saraiva et al., 2002a), neuritogenic (Mak et al., 2001) and vasodilator (Lin et al., 2005). In the present work we investigated the mechanism involved in the vasodilator effect induced by euxanthone in rat small mesenteric arteries. We demonstrate that euxanthone induces a concentration-dependent vasodilator effect by a mechanism dependent on the inhibition of calcium-sensitive protein kinase C.

Materials and methods

Animal experiments were performed according to the recommendations of the Brazilian Council for Animal Care and to the Ethics Committee of the Universidade Federal de Minas Gerais. Male Wistar rats were euthanized by decaptation. Small mesenteric arteries were mounted as previously described (Rezende et al., 2006). The presence of a functional endothelium was assessed by the ability of acetylcholine (ACh; 1 μM) to induce more than 70% relaxation of vessels pre-contracted with phenylephrine (3 μM). Euxanthone was added cumulatively during the tonic contractions induced by phenylephrine (3 μM), and KCl (80 mM) in tissues previously suspended in normal Krebs-Henseleit solution with the following composition (mM): NaCl 110.8, KCl 5.9, $NaHCO_3$ 25.0, $MgSO_4$ 1.07, $CaCl_2$ 2.49, NaH_2PO_4 2.33 and glucose 11.51. The vasodilator effect of euxanthone was expressed as percentage decrease in maximal contraction induced by the contractile agents, considering 100% relaxation as the point when the basal line was reached. Values of inhibitory concentration 50% (IC_{50}) were calculated graphically from concentration-response curves of euxanthone. In order to observe the effect of a single concentration of euxanthone (10 μM) in the calcium influx

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through the voltage-operated channels, this xanthone was added to the organ bath 15 min prior to the addition of KCl (80 mM) in vessels maintained in Ca^{2+}-free (without $CaCl_2$ plus 1.0 mM EGTA) solution followed by addition of 2.5 mM of $CaCl_2$. In this experimental condition, nifedipine (1 μM) was used as positive control. The same type of procedure was done in arteries maintained in Ca^{2+}-free solution and contracted with phenylephrine (10 μM) or phorbol 12-myristate 13-acetate (PMA; 10 μM), allowing to observe the effect euxanthone in the contractions dependent on the release of the intracellular calcium stores or on the activation of PKC, respectively. The contractile effect of PMA was also induced in arteries maintained in normal Krebs-Henseleit solution for PMA in the absence or in the presence of euxanthone (10 μM) added 15 min before PMA. Staurosporin (3 μM) was used as positive control for the mechanisms dependent on activation of PKC by PMA. Results are expressed as the mean ± s.e.m of five experiments. Student's *t*-test was used to analyze the results, and was considered significant when $P < 0.05$. Acetylcholine chloride, arachidonic acid, phenylephrine bitartrate, L-NAME, indomethacin, nifedipine, staurosporine and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma (USA). Spectroscopically pure euxanthone was isolated as described elsewhere (Santos et al., 2000), it was crystallized and 98% purity confirmed by HPLC. Euxanthone was solubilized in a mixture of distilled water/chremophor as a 10^{-1} M solution and diluted to the desired concentration with distilled water just before use. The final concentration of chremophor never exceeded 0.1%, which was without effect when exposed to control preparations.

Results and discussion

Euxanthone induced a concentration dependent relaxation in mesenteric arteries with a functional endothelium pre-contracted with phenylephrine (IC_{50} = 2.8 ± 0.3 μM; Fig. 2A). The relaxant effect induced by euxanthone was not modified by indomethacin or L-NAME (IC_{50} = 1.7 ± 0.4 μM and 2.4 ± 0.1 μM, respectively; Fig. 2A), although they were able to inhibit the contractions induced by arachidonic acid and the relaxations induced by ACh, respectively (not shown). These results suggest that the effect of euxanthone is not mediated by endothelial factors, such as, NO and cyclooxygenase-derived factors.

As illustrated in Fig. 2B, euxanthone also induced relaxations in mesenteric arteries with a functional endothelium pre-contracted with 80 mM KCl, with IC_{50} = 2.3 ± 0.4 μM. It suggests that euxanthone induces a vasodilator effect by inhibition of voltage-operated calcium channels, while the opening of potassium channels might not be involved. However, in arteries maintained at calcium-free solution and depolarized with 80 mM KCl, euxanthone (10 μM) poorly inhibited the contractions observed after addition of 2.5 mM of Ca^{2+} (Fig. 3A), while nifedipine (1 μM) almost completely abolished these contractions (Fig. 3A). This result suggests that other mechanism, rather than just inhibition of voltage-operated calcium channels, may be involved in the vasodilator effect of euxanthone.

In order to verify if the inhibition of the release of calcium from $InsP_3$-sensitive stores (Lagaud et al., 1999) would be involved in the vasodilator effect of euxanthone, the mesenteric arteries were pre-treated with this xanthone 15 min before addition of phenylephrine. In this experimental condition, euxanthone (10 μM) strongly inhibited the transient contractions induced by 10 μM phenylephrine (93.3 ± 3.8 %; $P < 0.001$ compared to control; not shown), suggesting that the inhibition of the release of calcium from $InsP_3$-sensitive stores or alterations in other calcium-sensitive mechanisms may be involved on the effect of euxanthone.

Finally we also investigated the relationship between the activation of PKC-dependent mechanisms and the vasodilator effect of euxanthone. The relevant role of PKC in the control of sustained contractions is well reported in the literature (Karaki et al., 1997). In arteries pre-contracted with PMA (10 μM), a PKC activator (Liu and Heckman, 1998), in the presence of extracellular calcium, euxanthone (10 μM) induced a remarkable inhibition (Fig. 3B) at a similar level as observed with staurosporine (3 μM). Conversely, in arteries maintained in absence of extracellular calcium and pre-contracted with PMA (10 μM), the inhibitory effect observed in the presence of euxanthone was weaker than that induced by staurosporine (Fig. 3C). As there was a strong difference between inhibitory effect of euxanthone when tissues were stimulated in normal or Ca^{2+}-free solution, it is possible that the inhibition of calcium entry modulated by PKC or of classical PKC isoforms may be involved in the mechanism of action of euxanthone.

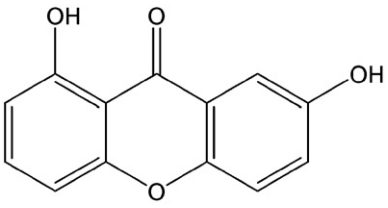

Fig. 1. Structure of Euxanthone.

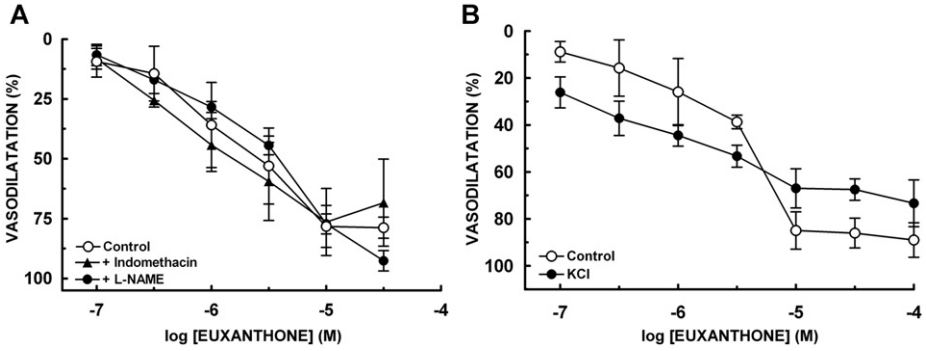

Fig. 2. Vasorelaxant effect euxanthone on rat small mesenteric artery with a functional endothelium. (A) Concentration-response curve of euxanthone on arteries pre-contracted with phenylephrine (3 M) in the absence or in the presence of indomethacin (10 M) or L-NAME (100 M). (B) Concentration-response curve of euxanthone on arteries pre-contracted with phenylephrine (control) or KCl (80 mM). All results are represented as mean ± s.e.m. of at least five experiments.

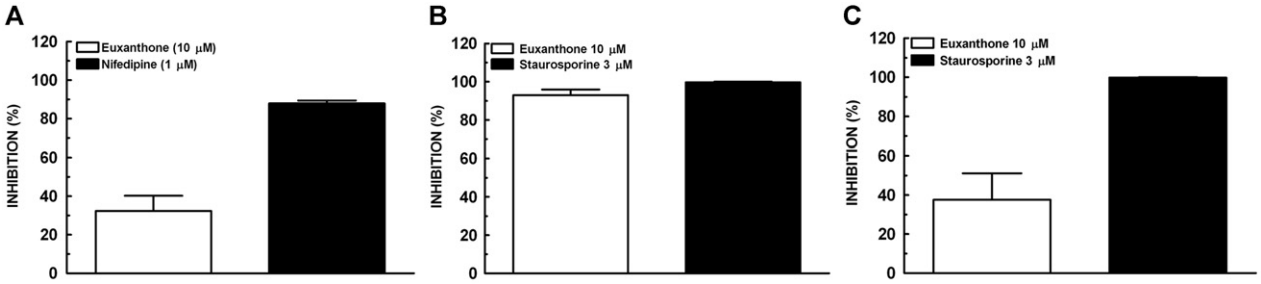

Fig. 3. Mechanism involved on the vasorelaxant effect of euxanthone. (A) Inhibitory effect of euxanthone and nifedipine on the contraction induced by addition of calcium (2.5 mM) in depolarized (KCl 80 mM) mesenteric arteries maintained in calcium-free physiological solution. (B) Inhibitory effect of euxanthone and staurosporine on mesenteric arteries contracted with PMA (10 μM) in physiological solution with calcium. (C) The same as in B in calcium-free physiological solution. All results are represented as mean ± s.e.m. of at least five experiments.

Therefore, euxanthone induces a vasorelaxant effect in rat resistance artery by inhibition of intracellular mechanisms dependent on calcium, most likely by inhibition of calcium-dependent PKC, rather than on influx of calcium by voltage-operated channels.

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