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(−)-α-Bisabolol inhibits preferentially electromechanical coupling on rat isolated arteries

R.J.B. de Siqueira *, H.V. Ribeiro-Filho, R.S. Freire, F. Cosker, W.B.S. Freire, A.A. Vasconcelos-Silva, M.A. Soares, S. Lahlou, P.J.C. Magalhães

Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil

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ABSTRACT

Previous findings enable us to hypothesize that (−)-α-bisabolol acts as inhibitor of voltage-dependent Ca^{2+} channels in smooth muscle. The current study was aimed at consolidating such hypothesis through the recording of isometric tension, measurement of intracellular Ca^{2+} as well as discovery of channel target using in silico analysis. In rat aortic rings, (−)-α-bisabolol (1–1000 μM) relaxed KCl- and phenylephrine-elicited contractions, but the IC_{50} differed significantly (22.8 [17.6–27.7] and 200.7 [120.4–334.6] μM, respectively). The relaxation of phenylephrine contractions remained unaffected by L-NAME, indomethacin, 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one, tetraethylammonium, glibenclamide or KT-5720. Under Ca^{2+}-free conditions, (−)-α-bisabolol did not alter the contractions evoked by phenylephrine or caffeine whereas it reduced those evoked by CaCl2 in KCl-, but not in PHE-stimulated preparations. Furthermore, it did not significantly alter the contractions evoked by phorbol 12,13-dibutyrate or induced by the extracellular Ca^{2+} restoration in cyclopiazonic acid-treated preparations. In mesenteric rings loaded with Fluo-4 AM, (−)-α-bisabolol blunted the tension and the cytosolic levels of Ca^{2+} in response to K^+ but not to norepinephrine. Silico docking analysis of the Cavβ2a subunit of voltage-dependent Ca^{2+} channel indicated putative docking sites for (−)-α-bisabolol. These findings reinforce the ability of (−)-α-bisabolol to inhibit preferentially contractile responses evoked by Ca^{2+} influx through voltage-dependent Ca^{2+} channels.

1. Introduction

(−)-α-Bisabolol, also known as levomenol, is a sesquiterpenic monocyclic alcohol abundantly found as the major constituent of the essential oil of chamomile (*Matricaria chamomilla* L., Asteraceae). It has been attributed to this compound the healing attributes of chamomile, the feature that argues for the use of (−)-α-bisabolol in pharmaceutical formulations in virtue of its anti-inflammatory and antiallergic properties [10]. In fact, it has been reported that (−)-α-bisabolol has protective effects against gastric damage caused by acetylsalicylic acid [12, 26]. The antitumor activity of (−)-α-bisabolol, which is mediated by its proapoptotic effects, has also recently received special attention in the literature [2–4].

In the cardiovascular system, intravenous injections of increasing doses of (−)-α-bisabolol induced dose-dependent hypotension and bradycardia in awake rats [13]. However, the mechanism of action of these cardiovascular effects has not been addressed. The hypotensive response to (−)-α-bisabolol could be mediated, at least partially, through an active vascular relaxation. We recently found that (−)-α-bisabolol induced endothelium-independent relaxation with a pharmacological potency that was higher in mesenteric than in aortic rings [5]. In only aortic rings, (−)-α-bisabolol relaxed KCl-induced contractions with pharmacological potency significantly higher than that observed in vessels contracted with phenylephrine. This data indicates that (−)-α-bisabolol counteracts contractile responses that preferentially recruit voltage-gated Ca^{2+} channels, similar to what was reported for tracheal smooth muscle preparations [5].

Putative Ca^{2+} channel blocking activity as the underlying mechanism for vasodilator action induced by (−)-α-bisabolol was previously demonstrated by Vuorela et al. [28]. Such activity has been also reported for bisabolol oxide and (+)-T-cadinol, two analogues of (−)-α-bisabolol, in experiments with isolated papillary muscle from guinea-pig hearts [16] and binding assays with dihydropyridine on the voltage-operated Ca^{2+} channels [30]. However, direct demonstration that (−)-α-bisabolol is able to interfere with

* Corresponding author at: Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará, R. Cel Nunes de Melo, 1127, Rodolfo Teófilo, 60430-270 Fortaleza, CE, Brazil. Tel.: +55 85 3366 8334; fax: +55 85 3366 8333.
E-mail address: rodrigo_siqueira@yahoo.com.br (R.J.B. de Siqueira).

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cytoplasmic Ca^{2+} dynamics was not demonstrated in smooth muscle cells hitherto.

Therefore, the present study was designed to test the hypothesis that pharmacological effects of (−)-α-bisabolol on vascular smooth muscle result from its ability to interfere with the intracellular levels of Ca^{2+}, especially on those events mediated electromechanically. Docking experiments and simultaneous measurement of mechanical force and $[Ca^{2+}]_i$ in Fluo-4 loaded mesenteric artery rings stimulated with either KCl or norepinephrine were performed to support the underlying mechanism involved in the vasorelaxant effects of (−)-α-bisabolol.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were obtained from our local colonies (vivarium of the Department of Physiology and Pharmacology, Federal University of Ceará) and maintained under constant temperature (22 ± 2 °C) with a 12 h light/12 h dark cycle and free access to food and water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85-23, revised 1996). All procedures described herein were reviewed by and had prior approval from the local animal ethics committee (Protocol n° 48/09 — CEPA).

2.2. Isolated ring-like aortic preparations

Rats were euthanized under sodium pentobarbital anesthesia and a segment of thoracic aorta was removed and immersed in perfusion medium at room temperature. After removing adhering fat and connective tissue, the aorta was cut transversally into cylindrical ring-like segments (1 × 5 mm) receiving careful transluminal insertion of steel wire triangular pieces (0.3 mm diameter) that allowed tissue suspension into 5-mL organ bath containing Krebs–Henseleit solution (continuously aerated at 37 °C with 5% CO_2 in O_2). Endothelium-containing preparations were stretched with a passive tension of 1 g and tension was recorded using isometric force transducer (ML870B60/C-V, AD Instruments, Australia) connected to a data acquisition system (PowerLab™ 8/30, AD Instruments). After an equilibration period of 60 min, control contractions were induced by adding a submaximal concentration (60 mM) of KCl to the bath. When two successive contractions showed similar amplitude, preparations were considered in equilibrium. At the beginning of the experiment, each aortic or mesenteric ring was precontracted with phenylephrine (0.1 μM) and thereafter challenged by acetylcholine (1 μM) to evaluate the integrity of endothelium. Preparations were considered to possess an intact endothelium when the vasorelaxant response to acetylcholine was 80% or greater. The concentration-effect curves were obtained by exposing the aortic preparation to cumulatively increasing concentrations of (−)-a-bisabolol (1–1000 μmol/L), which was added to the bath and maintained at a given concentration for 10 min.

2.3. Simultaneous measurement of force and intracellular Ca^{2+} in rings of mesenteric artery

Vessel segments from the second branch of the superior mesenteric artery were dissected and maintained in oxygenated Krebs–Henseleit solution at room temperature. After removing adhering fat under a microscope, cylindrical ring-like segments were obtained and carefully mounted in a confocal myograph chamber (DMT120CW Confocal Wire Myograph, Aarhus, Denmark) following the transluminal insertion of two tungsten wires (40 μm diameter). Then, under constant temperature (37 °C), a resting tension of 11.8 kPa was applied to each isolated artery segment. The preparation was incubated by 50 min with the fluorescent Ca^{2+} indicator Fluo-4 AM (5 μM) supplemented with pluronic acid (0.1% w/v) (Life Technologies, USA). After washing, the Ca^{2+} fluorescence was registered with an inverted confocal microscope (Olympus, IX81) using a 20× magnification and excitation/emission wavelengths of 488/505–515 nm. Sample rate was 1 frame/7 s and the intracellular Ca^{2+} variations were expressed in relative variation of the initial fluorescence (F/F_0). Simultaneously, the variations of the tension were recorded with an isometric force transducer connected to a data acquisition system (PowerLab™ 8/30, AD Instruments).

2.4. In silico docking analysis

We employed AutoDock 4.2 [14] in docking experiments using three-dimensional molecular structure of the ligand (−)-α-bisabolol (constructed by the molecular editor Avogadro 1.1.0; [6]) in pharmacophoric alignments with macromolecules based on two crystallographic models of a β-subunit isoform of voltage-gated L-type Ca^{2+} channel, the conserved core $Ca_v\beta_{2a}$ alone or in complex with the α-interaction domain (AID), namely AID-$Ca_v\beta_{2a}$ [27]. The macromolecules and ligand were prepared using the graphical interface AutoDockTools 1.5.6, which allowed the addition of polar hydrogens (Kollman) and partial charges (Gasteiger). For ligand, the number of active torsions was 5, and both macromolecules were considered rigid. Autogrid (part of the AutoDock package) allowed the construction of affinity grid fields, previously to the docking procedure. For all docking experiments, we used the genetic algorithm available in the software. In a first step of docking experiments, we constructed a 0.59 Å-spaced grid field that covered the entire macromolecule of either $Ca_v\beta_{2a}$ or AID-$Ca_v\beta_{2a}$. The numbers of energy evaluations and docking runs were 2,500,000 and 50, respectively, and other parameters were maintained as default. According to the results of the first step, a second series of simulations was performed by constructing two grid fields (with points spaced by 0.38 Å), one centered on the Hook domain (residues 121–169) and the other centered near to the conserved hydrophobic cleft named α-binding pocket. Such region engages $Ca_v\beta_{2a}$ with AID. The parameters were maintained as previously described. The cluster populations and binding energy as well as proximity of the residues were taken into account in the docking analysis.

2.5. Solutions and drugs

The perfusion medium used in isolated organ chamber was fresh modified Krebs–Henseleit solution (pH 7.4; in mM: 118.0 NaCl, 4.7 KCl, 1.18 KH_2PO_4, 1.18 $MgSO_4 \cdot 7H_2O$, 2.50 $CaCl_2$, 25.0 $NaHCO_3$, and 11.1 glucose). Nominally Ca^{2+}-free solution was prepared by omitting $CaCl_2$ and adding ethylene glycol bis(2-aminoethyl ether)-N,N, N′N′-tetraacetic acid (EGTA). All drugs were of analytical grade purity purchased from Sigma Co. (St. Louis, MO, USA). They were dissolved directly in Krebs–Henseleit solution except nifedipine, cyclopiazonic acid, KT-5720 and 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ) that were dissolved directly in DMSO (dimethyl sulfoxide). Solutions of (−)-α-bisabolol (bisabolol; Sigma-Aldrich) were prepared in Krebs–Henseleit solution containing Tween 80 (0.5%). Maximal percentage of Tween 80 in bath chamber was 0.06%. Substances were prepared as stock solutions and were brought to volume with Krebs–Henseleit solution in order to achieve a desired concentration in bath chamber.

2.6. Statistical analysis

Data are reported as mean ± SEM and n indicates the number of experiments. The IC_{50} values (i.e., the (−)-α-bisabolol concentration in μM that relaxes a contraction by 50%) were calculated by interpolation from semi-logarithmic plots and are expressed as geometric mean [95% confidence interval]. The significance ($p < 0.05$) of results was assessed using paired or unpaired Student's t-test, Mann–Whitney U-