European Journal of Pharmacology 669 (2011) 100–107

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

# Curine, a bisbenzylisoquinoline alkaloid, blocks L-type $Ca^{2+}$ channels and decreases intracellular $Ca^{2+}$ transients in A7r5 cells

Marcos A.A. Medeiros [a,b,1], José F. Pinho [c,1], Daysiane P. De-Lira [a], José M. Barbosa-Filho [a], Demetrius A.M. Araújo [a], Steyner F. Cortes [d], Virgínia S. Lemos [c,2], Jader S. Cruz [b,*,2]

[a] Laboratório de Tecnologia Farmacêutica (LTF), Universidade Federal da Paraíba, João Pessoa, PB, Brazil
[b] Departamento de Bioquímica e Imunologia, Laboratório de Membranas Excitáveis e Biologia Cardiovascular, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[c] Departamento de Fisiologia e Biofísica, Laboratório de Fisiologia Cardiovascular, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[d] Departamento de Farmacologia, Laboratório de Farmacologia Cardiovascular, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

ARTICLE INFO

*Article history:*
Received 23 December 2010
Received in revised form 13 July 2011
Accepted 30 July 2011
Available online 19 August 2011

*Keywords:*
Curine
Rat aorta
A7r5 cells
Vasodilation
L-type calcium channel

ABSTRACT

Curine is a novel bisbenzylisoquinoline alkaloid that has previously been reported as a vasodilator. The underlying mechanism(s) of the vasodilator effect of curine remains to be characterized. In this study, we investigated the cellular mechanism that is responsible for the vasodilator effect of curine in the rat aorta. The vasorelaxant activity of curine was recorded using a myograph. $Ca^{2+}$ currents in A7r5 cells were measured using the whole-cell patch-clamp technique. Intracellular $Ca^{2+}$ transients were determined using confocal microscopy. In a concentration-dependent manner, curine inhibited contractions elicited by high extracellular $K^+$ and Bay K8644 in the rat aorta and reduced the rise in the intracellular $Ca^{2+}$ concentration induced by membrane depolarization in response to an increase in extracellular $K^+$ concentration in vascular smooth muscle cells. Moreover, curine decreased the peak amplitude of L-type $Ca^{2+}$ currents ($I_{Ca,L}$) in a concentration-dependent manner without changing the characteristics of the current density vs. voltage relationship and the steady-state activation of $I_{Ca,L}$. Furthermore, curine shifted the steady-state inactivation curve of $I_{Ca,L}$ toward more hyperpolarized membrane potentials. None of the following modified the effect of curine on $I_{Ca,L}$ amplitude: 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterases; dibutyryl cyclic AMP, an activator of protein kinase A (PKA); or 8-Br-cyclic GMP, an activator of protein kinase G (PKG). Our results showed that curine inhibited the L-type voltage-dependent $Ca^{2+}$ current in rat aorta smooth muscle cells, which caused a decrease in intracellular global $Ca^{2+}$ transients that led to vasorelaxation.



## 1. Introduction

Voltage-gated $Ca^{2+}$ channels regulate electrical activity and many other intracellular processes, including smooth muscle contraction and relaxation. Calcium influx through voltage-gated L-type $Ca^{2+}$ channels ($Ca_V1.2$) is important for the control of vascular tonus, and its inhibition is associated with the antihypertensive effect that is produced by $Ca^{2+}$-channel blockers, such as verapamil, nifedipine and nitrendipine (Baker, 2000).

Dihydropyridines, such as nifedipine, can induce vasorelaxation and lower blood pressure by reducing peripheral vascular resistance. Unfortunately, there is a serious concern about their clinical use that has stimulated a number of research groups to develop novel $Ca^{2+}$ channel blockers based on lead compounds isolated from natural sources (Hill et al., 2001; Moosmang et al., 2003). Therefore, it is important to determine the pharmacological profile of these new drugs.

Bisbenzylisoquinoline alkaloids (BBA) have been implicated in the reduction of calcium influx (Liu et al., 1995). Curine (Fig. 1A) is the major structural BBA that is isolated from the root barks of *Chondrodendron platyphyllum*, a medicinal plant from the northwest region of Brazil (Barbosa-Filho et al., 2000). In a preliminary study, we demonstrated that curine causes vasodilatation in rat small mesenteric arteries, which provided the background for the further exploration of pharmacological mechanisms (Dias et al., 2002). To investigate whether the vasorelaxant effect of curine is dependent on the blockade of L-type $Ca^{2+}$ currents, we examined the possible interaction between curine and $Ca_v1.2$ using the patch-clamp technique. Additionally, we investigated the effects of curine on $Ca^{2+}$ handling by evaluating its effects on high $K^+$-induced $[Ca^{2+}]_i$ changes using Fluo4-based confocal $Ca^{2+}$ imaging in A7r5 vascular myocytes. Our

* Corresponding author. Tel.: +55 31 3409 2668; fax: +55 31 3409 2613.
*E-mail address:* jcruz@icb.ufmg.br (J.S. Cruz).
[1] Co-first authors.
[2] Co-senior authors.

0014-2999 © 2011 Elsevier B.V. Open access under the Elsevier OA license.
doi:10.1016/j.ejphar.2011.07.044

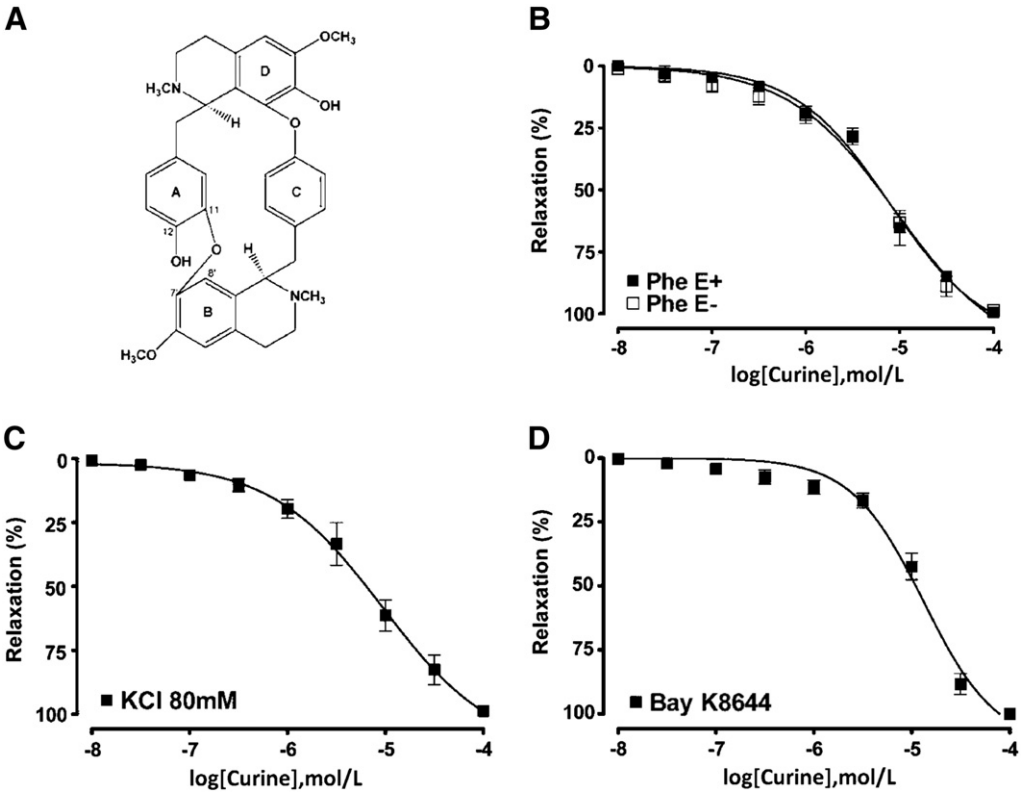


**Fig. 1.** (A) Chemical structure of curine. Molecular weight = 596. (B) Concentration–response curves for curine-induced relaxation in phenylephrine (0.3 μM) pre-contracted arteries in endothelium-containing (E+, closed squares, n = 5) and denuded endothelial (E−, open squares, n = 9) aortic rings. (C) Concentration–response curves for curine-induced relaxation in high $K^+$ (80 mM) pre-contracted arteries in denuded endothelium aortic rings (E−, closed squares, n = 7). (D) Concentration–response curves for curine-induced relaxation in BAY K8644 (100 nM) in denuded endothelium (E−) aortic rings bathed in Tyrode's solution supplemented with 20 mM $K^+$. The continuous lines were obtained by non-linear regression analysis using the logistic function.

results provide the first direct evidence that the relaxant effect of curine is likely associated with an impairment of $Ca^{2+}$ signaling.

## 2. Materials and methods

### 2.1. Animals

Twelve-week-old male Wistar rats were used in all experiments. Animals were housed, cared for and acclimatized in environmentally controlled quarters with a temperature maintained at 22 ± 1 °C and a 12/12 h light/dark cycle. Standard laboratory chow and drinking water were provided ad libitum. All principles of laboratory animal care were followed in accordance with the Animal Research Ethics Committee (CETEA) of the Federal University of Minas Gerais.

### 2.2. Preparation of isolated rat aortic rings

Descending thoracic aorta were prepared and mounted as described previously (Lemos et al., 2002). Briefly, animals were euthanized by decapitation, and their aorta were carefully removed and cleaned of connective and adipose tissues. Rings (2–3 mm long) were obtained, mounted in a 10 mL organ bath and maintained at 37 °C in Tyrode's solution (in mM): NaCl 158.3, KCl 4.0, $CaCl_2$ 2.0, $MgCl_2$ 1.05, $NaH_2PO_4$ 0.42, $NaHCO_3$ 10.0 and glucose 5.6. The solution was gassed with a 95% $O_2$ + 5% $CO_2$ mixture. The preparation was equilibrated under a resting tension of 1.0 g for at least 1 h. After the equilibration period, two contractile responses were evoked using submaximal concentrations of phenylephrine (0.3 μM). The presence of a functional endothelium was assessed by the ability of acetylcholine (10 μM) to induce more than an 80% relaxation of vessels that were pre-contracted with phenylephrine. In some experiments, the endothelial layer was removed by gently rubbing the intimal surface of the vessels with a cotton ball. The lack of acetylcholine relaxant activity indicated the absence of a functional endothelium. Isometric tension was recorded using a force-displacement transducer (FORT-10, WPI, Sarasota, FL, USA) connected to a pre-amplifier (Miobath-4, WPI, Sarasota, FL, USA).

### 2.3. Curine vasorelaxant activity in pre-contracted aortic rings

Vasorelaxant curine activity was measured in aortic rings with or without a functional endothelium that was pre-contracted with phenylephrine (0.3 μM; 6.71 ± 0.34 mN/mm for vessels with endothelium and 7.23 ± 0.39 mN/mm for endothelium-denuded vessels), KCl (80 mM; 8.3 ± 034 mN/mm) or BAY K8644 (100 nM; 6.8 ± 0.17 mN/mm). BAY K8644 evoked contractile tonic responses when the aorta preparations were incubated in Tyrode's solution supplemented with 20 mM $K^+$ (Usowicz et al., 1995). This maneuver is necessary because the binding of dihydropyridines depends on the channel state. Curine was added in increasing cumulative concentrations once contractile responses had reached steady-state. As a control for all of the above-mentioned protocols, another vessel segment from each rat was simultaneously monitored in the absence of drugs, and no reduction in contractile response was found. Results are expressed as percent decreases of the maximal contraction that was induced by the different smooth muscle contractile agents. Relaxation was calculated by comparing the developed tension before and after the addition of each concentration of curine, and the point when the baseline was reached was considered 100% relaxation. Values of 50% inhibitory concentration ($IC_{50}$) were calculated graphically from the individual concentration–response curves using non-linear regression.