Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 525 (2005) 123 – 127
www.elsevier.com/locate/ejphar

Ca^{2+}-dependent K^+ channels are targets for bradykinin B_1 receptor ligands and for lipopolysaccharide in the rat aorta

Nelson C. Farias, Teresa Feres, Antonio C.M. Paiva, Therezinha B. Paiva *

Department of Biophysics, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Botucatu, 862, 04023-062 São Paulo, SP, Brazil

Received 20 June 2005; received in revised form 20 September 2005; accepted 27 September 2005
Available online 2 November 2005

Abstract

Although rat aorta smooth muscle cells in culture constitutively express bradykinin B_1 receptors, the normotensive rat aorta does not respond to the bradykinin B_1 receptor agonist des-Arg^9-bradykinin, whereas vessels from the spontaneously hypertensive rat (SHR) respond to bradykinin B_1 receptor agonists with cell membrane hyperpolarization and relaxation. Bacterial lipopolysaccharide also is inactive on the normotensive rat but hyperpolarizes the SHR aorta. To determine whether this could be due to the increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in the SHR, we raised $[Ca^{2+}]_i$ in normotensive rats by treatment with thapsigargin. In the thapsigargin-treated aorta, both lipopolysaccharide and des-Arg^9-bradykinin induced hyperpolarization, which was reversed by the Ca^{2+}-dependent K^+ channel inhibitor iberiotoxin and by the bradykinin B_1 receptor antagonists Lys-[Leu^8]-des-Arg^9-bradykinin and [Leu^8]-des-Arg^9-bradykinin. Thus the bradykinin B_1 receptor, as well as lipopolysaccharide, needs activated Ca^{2+}-dependent K^+ channels for functional expression. The two bradykinin B_1 receptor inhibitors, however, have effects on Ca^{2+}-dependent K^+ channels which are not mediated by bradykinin B_1 receptors.

Keywords: Bradykinin B_1 receptor; Lipopolysaccharide; (Rat) aorta; Calcium-dependent K^+ channel

1. Introduction

Bradykinin B_1 receptors are not normally functional, becoming evident only after some types of tissue injury, such as the injection of bacterial materials or under pathological conditions such as inflammation and sepsis (Regoli and Barabe, 1980; McLean et al., 2000). Bradykinin B_1 receptor upregulation is due to de novo protein synthesis, in response to specific cytokines released in situations of trauma and stress (Passos et al., 2004; Marceau and Bachvarov, 1998). Agents that stimulate the synthesis of these cytokines, such as bacterial lipopolysaccharide, also lead to functional bradykinin B_1 receptor expression when administered either in vitro or in vivo (for a recent review see Leeb-Lunderberg et al., 2005).

In the case of the rat aorta, although smooth muscle cells in culture were shown to constitutively express functional bradykinin B_1 receptors (Schaeffer et al., 2001), isolated aorta preparations do not respond to bradykinin B_1 receptor agonists nor to lipopolysaccharide.

Lipopolysaccharide is known to activate large conductance Ca^{2+}-dependent K^+ (K_{Ca}) channels of vascular smooth muscle cell membranes, mediated by inducible nitric oxide synthase (iNOS), leading to hyporeactivity to vasoconstrictor agents (Thiemermann, 1997; Hecker et al., 1999; Muller et al., 2000). However, lipopolysaccharide can also activate K_{Ca} channels in the vascular smooth muscle independently of iNOS induction (Chen et al., 1999, 2000; Yakubovich et al., 2001).

We have previously shown that lipopolysaccharide (Farias et al., 2002), as well as the bradykinin B_1 receptor agonist des-Arg^9-bradykinin (Farias et al., 2004), induce dose-dependent hyperpolarization and relaxation in the aorta of the spontaneously hypertensive rat (SHR), but not in the normotensive Wistar rat. It was suggested that this difference may be due to the increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) present in the SHR (Jelicks and Gupta, 1990), since K_{Ca} channels are constitutively open in this strain (Liu et al., 1997). We have now explored this hypothesis by investigating whether increasing $[Ca^{2+}]_i$ would induce, in the normotensive rat aorta, a

* Corresponding author. Tel.: +55 11 55724583; fax: +55 11 55715780.
E-mail address: tbpaiva@biofis.epm.br (T.B. Paiva).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.09.047

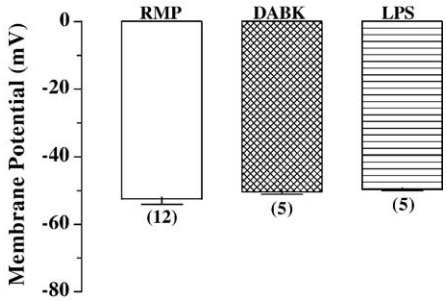

Fig. 1. Membrane potential measured in rat aortic rings. The resting membrane potential (RMP) and the effects of 1 μM des-Arg^9-bradykinin and 10 μg/ml lipopolysaccharide (LPS) are shown. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 10 cells were impaled, and the averages of the respective measurements were used to obtain the means±S.E.M.

behavior similar to that observed in the SHR vessels towards des-Arg^9-bradykinin and lipopolysaccharide. For this purpose we treated the rat aorta with thapsigargin, a selective inhibitor of the sarcoplasmic Ca^{2+}-ATPase (Thastrup et al., 1990) that promotes increase in $[Ca^{2+}]_i$ by emptying the intracellular Ca^{2+} stores. Iberiotoxin, an inhibitor of K_{Ca} channels, was used to demonstrate the role of these channels in the aortic smooth muscle responses, Lys-[Leu^8]-des-Arg^9-bradykinin and [Leu^8]-des-Arg^9-bradykinin were used as bradykinin B_1 receptor antagonists.

2. Materials and methods

2.1. Animals

Experiments were carried out using male normotensive Wistar rats from the Wistar Institute, Philadelphia, PA, USA, inbred at Escola Paulista de Medicina/Federal University of São Paulo, Brazil. The rats were 20–30 weeks old and weighed 250–350 g. They were killed by decapitation, their thoracic aortas were removed, cleaned of adherent connective tissue and cut into rings (3–4 mm length) for electrophysiological measurements. Care was taken to ensure that the endothelial layer was not damaged during tissue preparation. All procedures complied with the norms of the Ethics Committee for Research of the São Paulo Hospital of the Federal University of São Paulo.

2.2. Membrane potential

To observe the relaxant responses of the rat aorta to agonists, the preparations must be pre-contracted with vasoconstrictor agonists which inhibit K^+ channels (Quayle et al., 1997), blunting the relaxant response due the activation of these channels. To avoid this problem, intracellular microelectrodes were used to obtain direct measurement of the smooth muscle cell membrane potential in aortic rings (Nelson et al., 1990). These measurements yield very reproducible values with small deviations.

Micropipettes (borosilicate glass capillaries 1B120F-6, World Precision Instruments, WPI) were made by means of a horizontal puller (Model PN-3, Narishige, Tokio, Japan) and filled with 2 M KCl (tip resistance 20–40 MΩ and tip potential <6 mV). The microelectrodes were mounted in Ag/AgCl half-cells on a micromanipulator (Leitz, Leica) and connected to an electrometer (Intra 767, WPI).

The aortic rings were placed in a 2-ml perfusion chamber containing Krebs–bicarbonate solution of the following composition (in mM): NaCl 122, KCl 5.9, $MgCl_2$ 1.25, $NaHCO_3$ 15, $C_6H_{12}O_6$ 11, $CaCl_2$ 1.25 (pH 7.4). They were superfused at a rate of 3 ml min^{-1} with Krebs solution at 37 °C, aerated with the gas mixture 5% CO_2–95% O_2.

After an equilibration period of 2 h under an optimal resting tension of 1.0 g, the impalements of the smooth muscle cells were made from the adventitial side. The electrical signals were continuously monitored on an oscilloscope (Model 54645A, Hewlett Packard) and recorded in a potentiometric chart recorder (Model 2210, LKB-Produkter AB). The successful implantation of the electrode was evidenced by a sharp drop in voltage upon entry into a cell, a stable potential (±3 mV) for at least 1 min after impalement, a sharp return to zero upon exit, and minimal change (<10%) in microelectrode resistance after impalement.

Membrane potentials were measured before and after stimulation of the vessels with thapsigargin (1 μM), lipopolysaccharide (10 μg/ml) or des-Arg^9-bradykinin (1 μM), in the presence or absence of iberiotoxin (10 nM), Lys-[Leu^8]-des-Arg^9-bradykinin (10 μM) or [Leu^8]-des-Arg^9-bradykinin (10

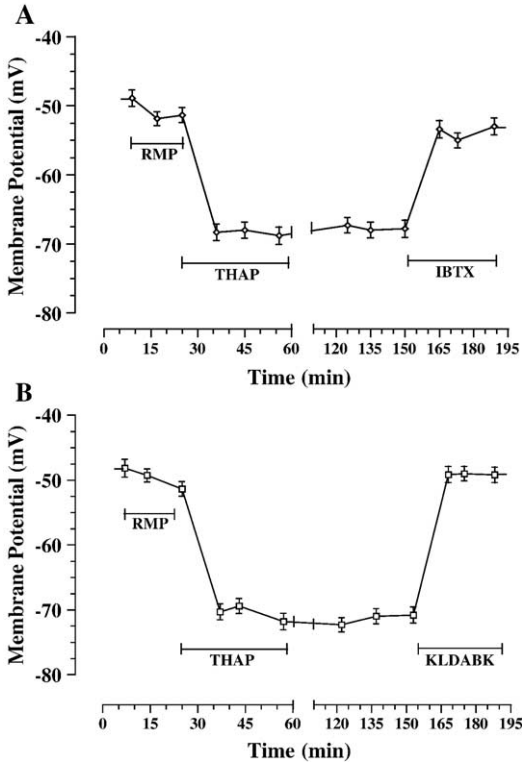

Fig. 2. Typical experiments in which, after recording the resting membrane potential (RMP), the rat aorta was superfused with 1 μM thapsigargin (THAP), causing a hyperpolarization that was not reversed by washing with Krebs solution. Superfusion with 10 nM iberiotoxin (IBTX) (A) or with 10 μM Lys-[Leu^8]-des-Arg^9-bradykinin (KLDABK) (B) caused depolarization to RMP levels.