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European Journal of Pharmacology 498 (2004) 163–169

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# Lys-[Leu$^8$,des-Arg$^9$]-bradykinin blocks lipopolysaccharide-induced SHR aorta hyperpolarization by inhibition of $Ca^{++}$- and ATP-dependent $K^+$ channels

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Received 27 November 2003; received in revised form 24 June 2004; accepted 5 July 2004
Available online 12 August 2004

**Abstract**

The mediators involved in the hyperpolarizing effects of lipopolysaccharide and of the bradykinin $B_1$ receptor agonist des-Arg$^9$-bradykinin on the rat aorta were investigated by comparing the responses of aortic rings of spontaneously hypertensive and normotensive Wistar rats. Endothelized rings from hypertensive rats were hyperpolarized by des-Arg$^9$-bradykinin and lipopolysaccharide, whereas de-endothelized rings responded to lipopolysaccharide but not to des-Arg$^9$-bradykinin. In endothelized preparations, the responses to des-Arg$^9$-bradykinin were inhibited by $N^{\omega}$-nitro-L-arginine and iberiotoxin. De-endothelized ring responses to lipopolysaccharide were inhibited by iberiotoxin, glibenclamide and $B_1$ antagonist Lys-[Leu$^8$,des-Arg$^9$]-bradykinin. This antagonist also inhibited hyperpolarization by des-Arg$^9$-bradykinin and by the á$_2$-adrenoceptor agonist, brimonidine. Our results indicate that $Ca^{2+}$-sensitive $K^+$ channels are the final mediators of the responses to des-Arg$^9$-bradykinin, whereas both $Ca^{2+}$- and ATP-sensitive $K^+$ channels mediate the responses to lipopolysaccharide. The inhibitory effects of Lys-[Leu$^8$,des-Arg$^9$]-bradykinin is due to a direct action on $Ca^{2+}$- and ATP-sensitive potassium channels.
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*Keywords:* Des-Arg$^9$-bradykinin; Lipopolysaccharide; Rat, spontaneously hypertensive; Aorta; $K^+$ channel

## 1. Introduction

The nonapeptide bradykinin and its biologically active metabolite [des-Arg$^9$]-bradykinin exert their effects by selective activation of two kinin receptor types: $B_1$ and $B_2$. The bradykinin $B_2$ receptor is constitutively expressed and mediates the majority of the visceral and vascular actions of bradykinin, whereas the bradykinin $B_1$ receptor is expressed mainly under pathological conditions such as inflammation and sepsis, being selectively activated by des-Arg$^9$ metabolites of the kinins (for reviews, see Regoli and Barabe, 1980; Mclean et al., 2000). Thus, kinins appear to be important in the pathogenesis of the systemic inflammatory response syndrome, or endotoxemia, caused by the release of lipopolysaccharide (LPS) from the outer membrane of gram-negative or gram-positive bacteria or fungi (Bone, 1994). Furthermore, endotoxemia is thought to be due partly to the release of nitric oxide (NO) by the action of NO synthase expressed in vascular smooth muscles (McCuskey et al., 1996).

Several findings have indicated that the bradykinin $B_1$ receptors are upregulated in endotoxemia. Thus, smooth muscles from LPS-treated animals show dose-dependent hypotensive responses to des-Arg$^9$-bradykinin (Marceau et al., 1983) and bradykinin $B_1$ receptor antagonists reverted the late phase of endotoxin-induced hypotension (Mclean et al., 1999). In addition, expression of the bradykinin $B_1$ receptor can be triggered by cytokines, interleukins and tumor necrosis factors, which are released by endotoxins (Regoli et al., 1981; Deblois et al., 1988; Campos et al., 1999).

The spontaneously hypertensive rat (SHR) is a good model to analyze the role of mediators involved in the responses of arterial smooth muscles to LPS and to bradykinin $B_1$ receptor agonists since this strain seems to

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doi:10.1016/j.ejphar.2004.07.002

have its own genetically determined immunoinflammatory response. Thus, Yen et al. (1997) observed a shorter survival time in SHR after LPS injection in comparison with normotensive rats. The higher mortality of SHR due to LPS was associated with overproduction of inducible NO synthase, which was harmful to these animals, since they already have higher levels of plasma nitrites (Wu and Yen, 1999).

Previous work showed that the normotensive rat aortic smooth muscle did not respond to the bradykinin $B_1$ receptor agonist des-Arg$^9$ bradykinin (DABK) (Schaeffer et al., 2001) nor to LPS (Farias et al., 2002). We have now found that the two agonists induce hyperpolarization in the SHR aorta, and in the present work, we investigate whether this difference in the behaviour of the two strains may be due to different states of their $Ca^{2+}$-dependent $K^+$ ($K_{Ca}$) channels.

The SHR aorta smooth muscle cells present an increased intracellular calcium concentration (Jelicks and Gupta, 1990), and consequently, their $K_{Ca}$ channels are more active than those of normotensive Wistar Kyoto (WKY) or Wistar (NWR) animals (Rusch et al., 1992; Fauaz et al., 2003). To determine whether this might be the reason for the different behaviours of NWR and SHR aortas when challenged with LPS or DABK, we compared the changes induced in the smooth muscle cell membrane potentials of aortic rings from the two strains by these two agonists and by $K^+$ channel antagonists. We also examined the hypothesis that $K_{Ca}$ channels mediate the effects of both DABK and LPS.

## 2. Materials and methods

### 2.1. Animals

Experiments were carried out using male spontaneously hypertensive rats (Okamoto and Aoki, 1963) and normotensive Wistar rats from the Wistar Institute, Philadelphia, PA, USA, inbred at Escola Paulista de Medicina, SP, Brazil. Normotensive Wistar rats were used rather than WKY rats since the latter strain's adequacy as normotensive controls are subject to doubt (Kurtz and Morris, 1987; Rapp, 1987) since SHR and WKY rats were shown to be genetically disparate (H'Doubler et al., 1991). The rats were 10–16 weeks old and their blood pressure values were 194±24 mm Hg for the SHR and 114±24 mm Hg for the normotensive rats.

The animals were killed by decapitation to remove their thoracic aorta, which were cleaned of adherent connective tissue and cut into rings (3–4 mm length) for the electrophysiological measurements. Care was taken to ensure that the endothelial layer was not damaged during tissue preparation. In some rings, the endothelium was removed by gentle rubbing of the intimal surface with a plastic tube wrapped in cotton. All procedures complied with the norms of the Ethic Committee for Research of the São Paulo Hospital/Federal University of São Paulo.

### 2.2. Membrane potential

Instead of measuring the relaxation of aortas precontracted by vasoconstrictors (e.g., noradrenaline, endothelin, angiotensin II), in our study, we used direct measurements of the vascular smooth muscle membrane potential to avoid interference from the effects of those agonists in our measurements (Nelson et al., 1990).

Micropipettes were made from borosilicate glass capillaries (1B120F-6, World Precision Instruments, WPI) by means of a horizontal puller (Model PN-3, Narishige, Tokyo, 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 animals were decapitated and bled, and the thoracic aorta was removed and placed in 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). Rings of 1-cm length were cut and placed in a 2-ml perfusion chamber and superfused, at a rate of 3 ml min$^{-1}$ with Krebs solution (pH 7.4; 37 °C), aerated with a mixture 5% of $CO_2$–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). 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 LPS (10 μg ml$^{-1}$), des-Arg$^9$-bradykinin (1 μM) or UK 14,304 [5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2yl)-6-quinoxalin-amine] (1 nM) in the presence or absence of $N^{\omega}$-nitro-L-arginine (L-NNA) (50 μM), iberiotoxin (10 nM) or Lys-[Leu$^8$]-des-Arg$^9$-bradykinin (KLDABK) (10 μM). The contact of the drugs with the preparations before the impalements was 10 min. The presence of a functional endothelium was tested in all preparations by checking the response to acetylcholine (10 μM) which is characteristic of vessels with an intact endothelium (Furchgott, 1981).

### 2.3. Drugs

L-NNA, LPS (*Escherichia coli* lipopolysaccharide, 0111:B4), iberiotoxin, acetylcholine chloride and cromakalin were purchased from Sigma (St. Louis, MO, USA). UK 14,304 was purchased from Research Biochemicals International (Natik, MA, USA). Des-Arg$^9$-bradykinin and Lys-[Leu$^8$]-des-Arg$^9$-bradykinin were purchased from

Bachem Bioscience (King of Prussia, PA, USA). The inorganic salts were products of the highest analytical grade from Merck (Darmstadt, Germany). Maximally effective concentrations of the drugs, as determined by concentration–response curves (see Fig. 2B and Fauaz et al., 2000), were used.

### 2.4. Statistical analysis

All data are expressed as means±S.E.M. with the number of animals in parentheses. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the Newman–Keuls test in the case of pairwise comparisons between groups. When the data consisted of repeated observations at successive time points, ANOVA for repeated measurements was applied to determine differences between groups. When more than one impalement was made on the same aortic ring from the same rat, the measurements were averaged and considered as $n$=1. Differences were considered significant when $P$<0.05.

## 3. Results

The resting potential of the SHR aortic rings was found to be more negative in the SHR (−69.7±0.8 mV) than in the normotensive rat (−52.44±0.9), in agreement with Fauaz et al. (2003), who attributed this relative hyperpolarized state of the SHR vessels to the increased numbers of $K_{Ca}$ channels which are constitutively open in this strain.

Although the presence of constitutive bradykinin $B_1$ receptors has been demonstrated in aortic smooth muscle cells of normotensive rats (Schaeffer et al., 2001), Fig. 1A shows that neither the $B_1$ agonist des-Arg$^9$-bradykinin nor LPS had an effect on the membrane potential of normotensive rat endothelized (E+) aortic rings. Fig. 1B shows that the response of this preparation to acetylcholine was partially inhibited by the ATP-dependent $K^+$ ($K_{ATP}$) channel inhibitor glibenclamide and totally abolished by the NO synthase inhibitor $N^{\omega}$-nitro-L-arginine (L-NNA).

In the SHR, however, both des-Arg$^9$-bradykinin and LPS induced significant hyperpolarizing responses in E+ rings, whereas in de-endothelized (E−) rings, the responses were induced by LPS but not by des-Arg$^9$-bradykinin (Fig. 2A). To evaluate the participation of NO in the responses of SHR E+ rings to des-Arg$^9$-bradykinin, stimulation with the agonist was performed in the presence of L-NNA. This NO synthase inhibitor blocked the hyperpolarization elicited by des-Arg$^9$-bradykinin (Fig. 3), indicating that this response is mediated by NO release by the endothelium.

The relaxant effect of NO on vascular smooth muscles was shown to result from a direct action on $K_{Ca}$ channels (Bolotina et al., 1994; Murphy and Brayden, 1995; Taguchi et al., 1996), which are present in increased numbers in the SHR (Liu et al., 1997) but not in the normotensive rat (Silva et al., 1994; Fauaz et al., 2000). Therefore, the $K_{Ca}$ channel inhibitor iberiotoxin was used to determine their role in the responses of the SHR aortic rings to des-Arg$^9$-bradykinin, to LPS and to acetylcholine. Fig. 4 shows that iberiotoxin, by itself, caused membrane depolarization, suggesting that $K_{Ca}$ channels are constitutively activated in the SHR aorta. The response of these rings induced by des-Arg$^9$-bradykinin was completely inhibited by iberiotoxin, indicating that $K_{Ca}$ channels mediate these responses. In the case of LPS, a partial but significant inhibition by iberiotoxin was observed, in agreement with a previous report that LPS acts on both $K_{Ca}$ and $K_{ATP}$ channels (Farias et al., 2002). A partial inhibition of acetylcholine was also caused by iberiotoxin (Fig. 4), indicating that this agonist also acts on $K_{ATP}$ channels (see Fig. 1B).

To verify the role of bradykinin $B_1$ receptor activation in the hyperpolarizing responses to des-Arg$^9$-bradykinin, to LPS and to acetylcholine, SHR E+ rings were stimulated in the presence of the bradykinin $B_1$ receptor antagonist KLDABK. Fig. 5 shows that KLDABK, by itself, also

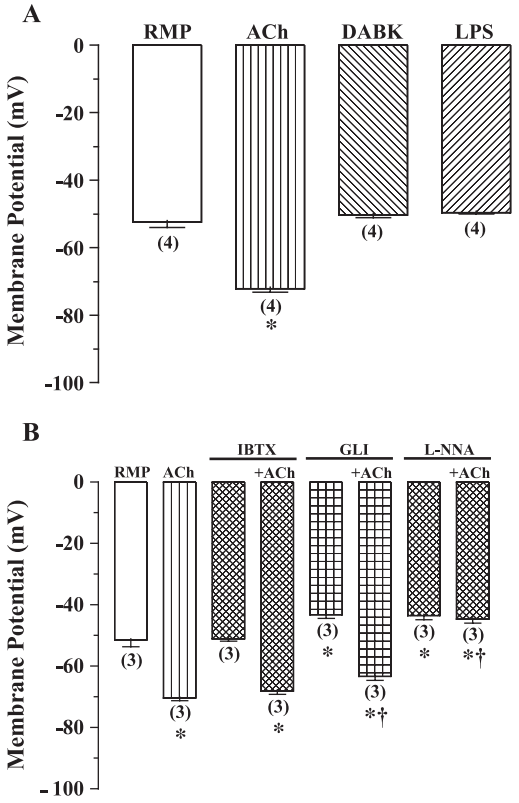


Fig. 1. Membrane potentials measured in E+ aortic rings from normotensive Wistar rats. (A) Effects of 10 μM acetylcholine (ACh), 1 μM des-Arg$^9$-BK (DABK) and 10 μg ml$^{-1}$ LPS. (B) Acetylcholine (ACh), 10 μM, in the absence or in the presence of 10 nM iberiotoxin (IBTX), or 1 μM glibenclamide (GLI) or 50 μM L-NNA. RMP, resting membrane potential. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the means and s.e. means. *$P$<0.05 versus respective RMP. †$P$<0.05 versus the response to ACh (Newman–Keuls test).

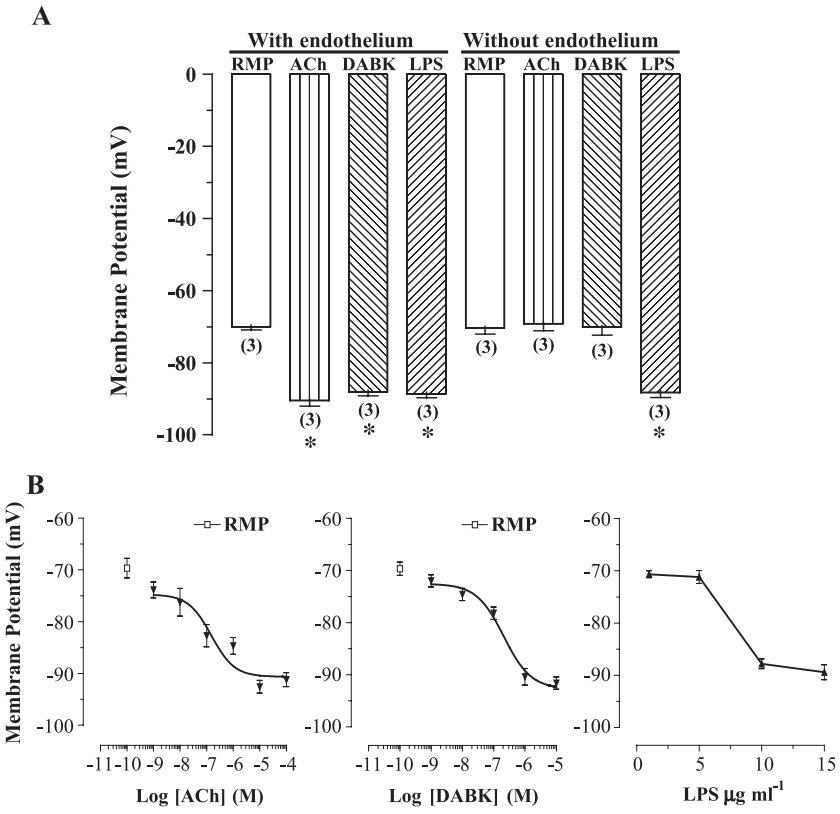


Fig. 2. (A) Membrane potential measured in E+ and E− aortic rings from SHR. The resting membrane potential (RMP) and the effects of 10 μM acetylcholine (ACh), 1 μM des-Arg$^9$-BK (DABK) and 10 μg ml$^{-1}$ LPS are shown. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the means and s.e. means. *$P$<0.05 versus respective RMP (Newman–Keuls test). (B) Dose–response curves for the effects of acetylcholine (ACh), DABK and LPS on the membrane potential of SHR E+ aortic rings, showing that maximally effective doses of the agonists were used in the measurements shown in panel A. Data are averages and s.e. means of 4–7 measurements.

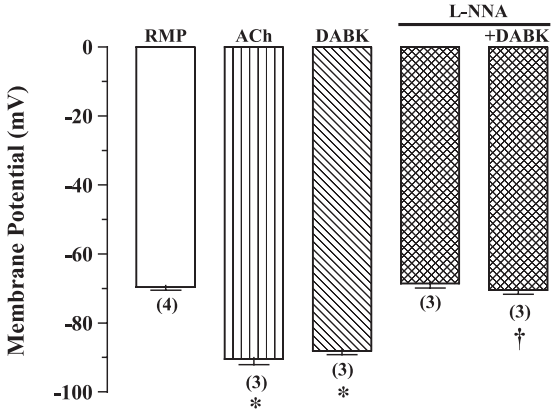


Fig. 3. Membrane potential measured in E+ aortic rings from SHR. The resting membrane potential (RMP) and the effects of 10 μM acetylcholine (ACh), 1 μM des-Arg$^9$-bradykinin (DABK) and 50 μM L-NNA (in the absence or in the presence of DABK) are shown. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the means and s.e. means. *$P$<0.05 versus respective RMP. $^{\dagger}P$<0.05 versus the response to DABK (Newman–Keuls test).

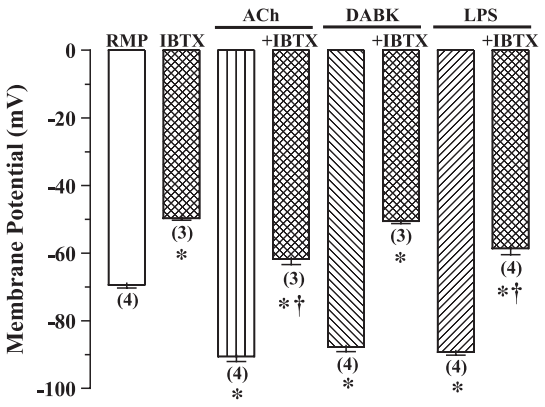


Fig. 4. Membrane potential measured in E+ aortic rings from SHR. The resting membrane potential (RMP) and the effects of 10 nM iberiotoxin (IBTX) in the absence or in the presence of 10 μM acetylcholine (ACh), 1 μM des-Arg$^9$-bradykinin (DABK) or 10 μg ml$^{-1}$ LPS are shown. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the means and s.e. means. *$P$<0.05 versus respective RMP. $^{\dagger}P$<0.05 versus the response to iberiotoxin (Newman–Keuls test).

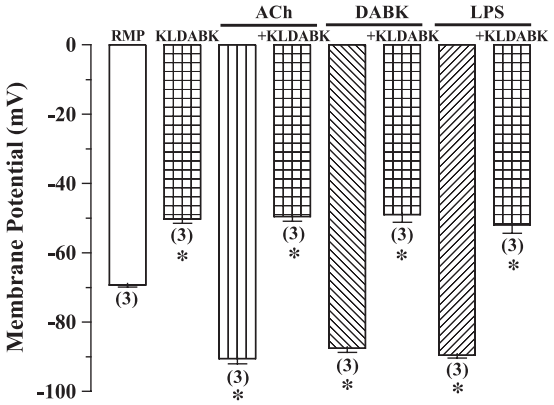


Fig. 5. Membrane potential measured in E+ aortic rings from SHR. The resting membrane potential (RMP) and the effects of 10 μM KLDABK, in the absence or in the presence of 10 μM acetylcholine (ACh), 1 μM des-Arg$^9$-bradykinin (DABK) or 10 μg ml$^{-1}$ LPS are shown. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the means and s.e. means. *$P$<0.05 versus respective RMP (Newman–Keuls test).

caused membrane depolarization, and the response to des-Arg$^9$-bradykinin was completely abolished by this inhibitor. Surprisingly, the responses induced by LPS and acetylcholine were also blocked by KLDABK, suggesting that this compound may not be a specific bradykinin $B_1$ receptor antagonist and behaves as an inhibitor of both $K_{ATP}$ and $K_{Ca}$ channels.

In SHR E− rings, KLDABK also caused depolarization and completely blocked the hyperpolarization induced by LPS (Fig. 6). This inhibitory effect might be at the level of $K_{Ca}$ channels since the responses were also inhibited by iberiotoxin (Fig. 4). To further investigate this possibility, we used UK 14,304, an $\alpha_2$ adrenergic agonist previously

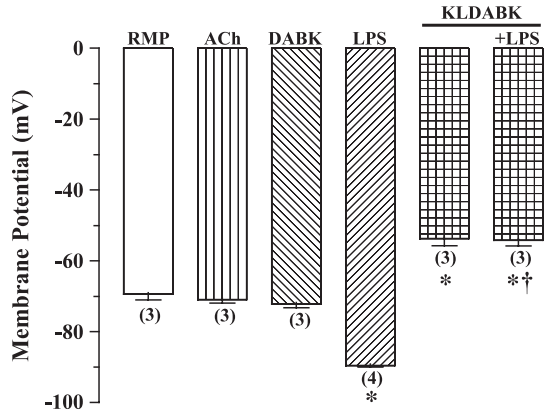


Fig. 6. Membrane potential measured in E− aortic rings from SHR. The resting membrane potential (RMP) and the effects of 10 μM acetylcholine (ACh), 1 μM des-Arg$^9$-bradykinin (DABK), 10 μg ml$^{-1}$ LPS and 10 μM KLDABK (in the absence or in the presence of LPS) are shown. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the means and s.e. means. *$P$<0.05 versus respective RMP. †$P$<0.05 versus the response to LPS (Newman–Keuls test).

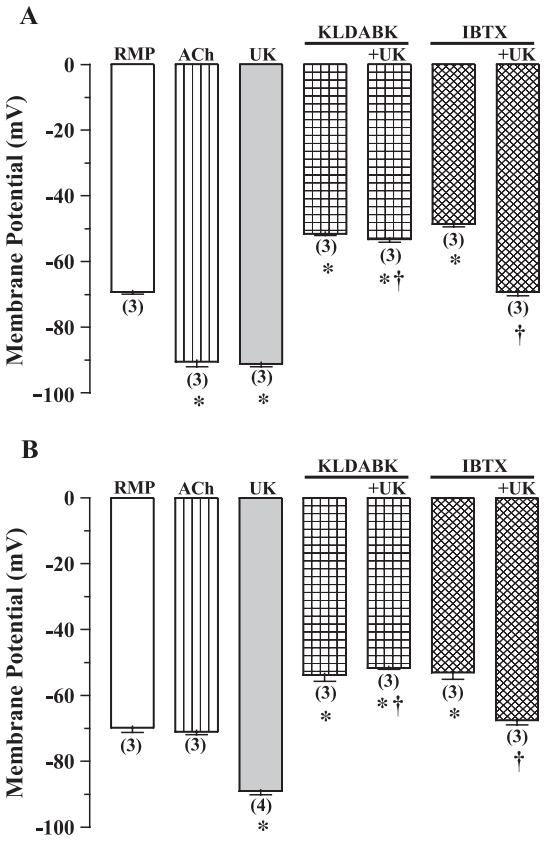


Fig. 7. Membrane potential measured in SHR aortic rings with (A) or without (B) endothelium. The effects of 10 μM acetylcholine (ACh) and 1 nM UK 14,304 (in the absence or in the presence of 10 μM KLDABK or 10 nM iberiotoxin (IBTX). RMP, resting membrane potential. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the mean and s.e. mean. *$P$<0.05 versus RMP. †$P$<0.05 versus UK 14,304 (Newman–Keuls test).

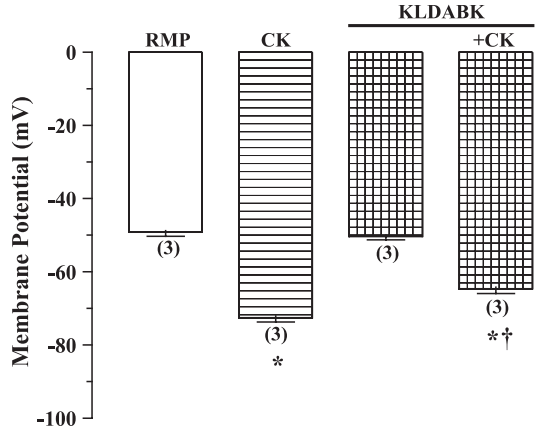


Fig. 8. Membrane potential measured in E+ aortic rings from normotensive Wistar rats. The resting membrane potential (RMP) and the effects of 1 μM cromakalin (CK), in the absence or in the presence of 10 μM KLDABK, are shown. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5–12 cells were impaled, and the averages of the respective measurements were used to obtain the means and s.e. means. *$P$<0.05 versus respective RMP (Newman–Keuls test). †$P$<0.05 versus the response to CK (Newman–Keuls test).

shown to hyperpolarize aortic SHR E− rings by opening $K_{Ca}$ and $K_{ATP}$ channels (Fauaz et al., 2003). Fig. 7 shows that both E− and E+ SHR aortic rings responded to UK 14,304 with hyperpolarization which was completely abolished by KLDABK and partially inhibited by iberiotoxin, confirming that this compound may act by blocking $K_{Ca}$ and $K_{ATP}$ channels.

To ascertain that KLDABK also inhibits $K_{ATP}$ channels, we determined its effect on the responses to a specific agonist for these channels, namely, cromakalin. This was done in the normotensive rat aorta where $K_{ATP}$ channels predominate. Fig. 8 shows that KLDABK caused a significant inhibition of the normotensive rat aortic rings to cromakalin.

## 4. Discussion

Although the bradykinin $B_1$ receptors are thought to be expressed in living animals only after exposure to noxious stimuli (Regoli et al., 1981), they were shown to be constitutive in rat aorta cultured smooth muscle cells (Schaeffer et al., 2001). However, the $B_1$ agonist des-Arg$^9$-bradykinin was ineffective in normotensive rat aortic rings (Fig. 1A), indicating that in these vessels, the receptor may be present but are not functional.

As expected, acetylcholine caused hyperpolarization in normotensive rat rings, which was completely abolished by L-NNA, indicating that NO is responsible for this response, probably by increasing cGMP (Rapoport and Murad, 1983) which stimulates the Ca-ATPase in the plasma membrane and in the sarcoplasmic reticulum (Taylor et al., 1999). These effects cause reduction of cytoplasmic $Ca^{2+}$ concentration, leading to hyperpolarization and relaxation of the smooth muscle. Activation of $K_{Ca}$ or $K_{ATP}$ channels does not play a significant role in the responses of normotensive rat rings to acetylcholine since iberiotoxin and glibenclamide had only very small effects on these responses (Fig. 1B). The lack of effect of LPS on normotensive rat rings agrees with previously published evidence that this agonist acts through $K_{Ca}$ channels (Farias et al., 2002) which appear to be less operative in that strain (England et al., 1993).

In the SHR, des-Arg$^9$-bradykinin caused a significant hyperpolarization in E+, suggesting that bradykinin $B_1$ receptors are functional in these animals, but not in E− aortic rings (Fig. 2A). Bradykinin $B_1$ receptor-mediated vasodilation responses are usually endothelium-dependent, as described in several vascular smooth muscles (Mclean et al., 2000). The response of SHR E+ aortic rings to des-Arg$^9$-bradykinin was inhibited by L-NNA (Fig. 3), indicating that $B_1$ agonists may act by activation of NO synthase in the endothelium and subsequent release of NO. The effect of des-Arg$^9$-bradykinin was blocked by iberiotoxin (Fig. 4), indicating that the responses to this agent are mediated by $K_{Ca}$ channels. Since the relaxant effect of NO on vascular smooth muscles was shown to result from a direct action on $K_{Ca}$ channels (Bolotina et al., 1994; Murphy and Brayden, 1995; Taguchi et al., 1996), we conclude that the effect of $B_1$ agonists on the SHR aorta is mediated by NO release from the endothelium which causes opening of these channels in the smooth muscle cells.

Lipopolysaccharide did not elicit a response in the normotensive rat, but caused hyperpolarization in SHR aortic rings. However, in contrast to the effect of des-Arg$^9$-bradykinin, the responses to LPS were not affected by the absence of a functional endothelium indicating that LPS acts on the SHR through a direct effect on the smooth muscle $K_{Ca}$ channels. Furthermore, the responses of E+ and E− preparations were partially inhibited by iberiotoxin (Fig. 4) and totally blocked by iberiotoxin plus glibenclamide (Farias et al., 2002), indicating that both the $K_{Ca}$ and the $K_{ATP}$ channels are involved in the responses to LPS. The effect of acetylcholine in SHR aorta was also partially inhibited by iberiotoxin (Fig. 4) and totally blocked by iberiotoxin plus glibenclamide (not shown), indicating the involvement of both $K_{Ca}$ and $K_{ATP}$ channels in these responses.

Surprisingly, the bradykinin $B_1$ receptor antagonist KLDABK blocked not only the responses of the SHR E+ aortic rings to des-Arg$^9$-bradykinin, but also those elicited by LPS and by acetylcholine (Fig. 5). Furthermore, the effect of LPS in SHR E− rings was totally blocked by KLDABK (Fig. 6), indicating that this agent inhibits the opening of $K_{Ca}$ and $K_{ATP}$ channels in the smooth muscle.

Another interesting observation is that both the inhibition of $K_{Ca}$ channels by iberiotoxin (Fig. 4) and the effect of bradykinin $B_1$ receptor antagonist KLDABK (Fig. 5) induced significant depolarizations of the SHR aorta smooth muscle membrane. This indicates that the two inhibitors induce closing of $K_{Ca}$ channels, which were proposed to be constitutively open in the SHR aorta, leading to their relatively hyperpolarized state in relation to the normotensive rat. The hyperpolarizing effect of agonists could be due to these agents increasing the open state probability of the channels.

To better evaluate the possibility that KLDABK may act directly on $K_{Ca}$ channels, we studied the effect of UK 14,304, an $\alpha_2$ adrenergic agonist that was shown to open these channels in the SHR aorta (Fauaz et al., 2003). The hyperpolarizing response induced by this agonist was inhibited by both iberiotoxin and KLDABK, confirming the hypothesis that KLDABK has a direct action on both $K_{Ca}$ and $K_{ATP}$ channels. A direct effect of KLDABK on $K_{ATP}$ channels was also demonstrated by the fact that cromakalin, a specific activator of these channels, was partially inhibited by KLDABK (Fig. 8). This should be taken into account when using KLDABK as a supposedly specific bradykinin $B_1$ receptor antagonist.

In conclusion, our results indicate that:

(1) LPS and des-Arg$^9$-bradykinin hyperpolarize SHR but not normotensive rat aortic rings. The response to DABK is endothelium-dependent and is due to NO activation of $K_{Ca}$ channels in the smooth muscle. The

SHR aorta response to LPS is not dependent on the endothelium and is due to a direct action on $K_{Ca}$ and $K_{ATP}$ channels in the smooth muscle.

(2) The endothelium-dependent hyperpolarizing effect of acetylcholine in the normotensive rat is not due to activation of $K^+$ channels and is probably mediated by the NO–cGMP–CaATPase pathway. In the SHR, acetylcholine induces an endothelium-dependent hyperpolarization by NO-induced activation of both $K_{Ca}$ and $K_{ATP}$ channels.

(3) The bradykinin $B_1$ receptor inhibitor KLDABK blocked the hyperpolarizing responses induced by all the agonists by a direct action on $K_{Ca}$ and $K_{ATP}$ channels.

## Acknowledgements

We are grateful to Nelson Mora for competent technical assistance. This work was supported by grants and fellowships from the São Paulo State Research Foundation (FAPESP), the Brazilian National Research Council (CNPq) and CAPES.

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