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Superoxide anion and K^+ channels mediate electrical stimulation-induced relaxation in the rat basilar artery

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Abstract

Electrical field stimulation (a single pulse, 0.2 ms) caused a rapid relaxation of rat basilar artery segments precontracted with different agents, but not with 30 mM KCl. This relaxation was not modified by endothelium removal, 10 μM tetrodotoxin, 1 μM propranolol, 1 μM atropine, 30 μM indomethacin, 10 μM methylene blue, 100 μM N^G-nitro-L-arginine methyl ester or 1 μM cimetidine but it was significantly reduced by 50 and 100 U/ml superoxide dismutase. Charybdotoxin (0.1 and 0.2 μM), a blocker of large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}), decreased the relaxation elicited by electrical stimulation, whereas it was unaltered by 10 μM glibenclamide or 1 μM apamin, blockers of ATP-sensitive (K_{ATP}) or small-conductance K_{Ca} channels, respectively. Thapsigargin (0.01 and 0.1 μM), an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, increased the electrical stimulation-induced relaxation, which was nearly abolished by charybdotoxin. These results show that electrical stimulation induces endothelium-independent and non-neurogenic relaxations in the rat basilar artery. This response appears to involve generation of superoxide anion, increase of cytosolic free Ca^{2+} concentration and subsequent activation of BK_{Ca} channels.

Keywords: Basilar artery; Electrical stimulation; Superoxide anion; K^+ channel

1. Introduction

Relaxation of precontracted blood vessels in response to electrical field stimulation has been observed in some arteries (Ebeigbe et al., 1983; Axelsson et al., 1989; Hardebo et al., 1989; González and Estrada, 1991). This vasodilator response may be mediated by different agents or mechanisms, such as noradrenaline release and subsequent activation of β-adrenoceptors (Cohen et al., 1983), neurogenic release of nitric oxide (González and Estrada, 1991) or calcitonin-gene related peptide (Kawasaki et al., 1988), direct smooth muscle hyperpolarization (Kotecha and Neild, 1988), histamine receptor activation (Ebeigbe et al., 1983) and generation of oxygen free radicals (Hardebo et al., 1989).

Cerebral arteries are densely innervated, containing adrenergic, cholinergic, nitregic and peptidergic nerves, which participate in the control of cerebrovascular tone (Edvinsson et al., 1993; Tomimoto et al., 1993; Matthew and Wadsworth, 1994). Neurogenic (González and Estrada, 1991; Matthew and Wadsworth, 1994), and non-neurogenic (Hardebo et al., 1989) vasodilations induced by electrical stimulation in these arteries, mediated by nitric oxide and free radicals, respectively, has been reported. However, the role of cholinergic nerves in this relaxation seems to be controversial (Van Riper and Bevan, 1992).

The role of the reactive oxygen species, superoxide anion, hydrogen peroxide and hydroxyl radical, on the regulation of the cerebrovascular tone has been scarcely studied. They participate in cerebral ischemia-reperfusion injury (Kinouchi et al., 1991; Cao and Phillis, 1994) and are generated in cerebral vascular damage (Kontos et al., 1983). In addition, they are involved in the relaxation caused by electrical stimulation (Hardebo et al., 1989) and vasodilator agents as bradykinin (Sobey et al., 1997) in cerebral arteries. Free radicals are toxic for the cells (Kontos et al., 1983; Marín and Rodríguez-Martínez, 1995), and those produced by electrical stimulation may damage cerebrovascular cells (Lamb et al., 1987; Hardebo et al., 1989). Lately, it has been reported that superoxide anion is able to produce relaxation in cat pial arteries using cranial

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windows (Wei et al., 1996). It is possible that non-neurogenic vasodilations elicited by electrical stimulation are mediated by generation of oxyradicals, and these in turn, may induce K^+ channel activation, a mechanism that produces hyperpolarization and closing of membrane Ca^{2+} channels (Kitazono et al., 1993; Nelson and Quayle, 1995). In addition, hyperpolarization appears to be a major mechanism for dilatation of cerebral blood vessels (Brian et al., 1996).

The present study was undertaken in the rat basilar artery to assess the ability of electrical stimulation to induce vasodilator responses, and then to ascertain the involvement of noradrenaline, nitric oxide, acetylcholine and oxyradicals in these responses, as well as the mechanism involved, particularly the participation of K^+ channels. The results obtained suggest that electrical stimulation induces endothelium-independent and non-neurogenic relaxations in great part mediated by superoxide anion that stimulates Ca^{2+}-activated K^+ channels (K_{Ca}).

2. Materials and methods

Animal care and use followed the directions of the European Community guidelines (Real Decreto 223/88, March 14, Spain).

2.1. Isolation of small cerebral arteries

Male, 12-week-old Wistar rats were killed by CO_2 inhalation. The brain was quickly removed and placed in Krebs–Henseleit solution at room temperature. The basilar artery was dissected out, cleaned of connective tissue under microscope and divided into rings of 2 mm in length.

Rings were set up in a myograph (JP-Trading, Denmark) for measuring isometric tension development, as described by Mulvany and Halpern (1977). Briefly, two 40-μm stainless-steel wires were passed through the lumen of the ring and fixed on support jaws on each side. One jaw was connected to a micrometer screw, by which the diameter of the arterial lumen could be varied, and the second jaw was attached to a force transducer connected to a polygraph (Letica, Poly-Graph 4000). The arteries were allowed to equilibrate under zero tension in Krebs–Henseleit solution at 37°C pH 7.4 and gassed with 5% carbon dioxide in oxygen. A passive diameter–tension curve of each ring was determined by stretching in stepwise increments of passive tension. The arteries were then set to an internal circumference equivalent to 90% of they would have when relaxed in vivo under a transmural pressure of 100 mm Hg (13.3 KPa). Then, to assess the ability of segments to develop contractile response, they were contracted twice, at a 10-min interval, in 125 mM K^+-substituted physiologic salt solution.

2.2. Determination of the presence of endothelium

The presence and functionality of the vascular endothelium were checked by the ability of acetylcholine (10 μM) to induce relaxation in segments precontracted with prostaglandin $F_{2\alpha}$ (10 μM). In some arteries, endothelium was mechanically removed by gentle rubbing of the intimal surface with a stainless steel wire (40 μm diameter) inserted through the lumen. The failure of segments to relax upon the addition of acetylcholine indicated that endothelium had been successfully removed.

2.3. Electrical stimulation

The arterial segments were placed between platinum plate electrodes of 1.5 mm in length, positioned on either side of the ring, 1.5 mm apart. The stimulation parameters used were a single square-wave pulse (200 mA, 0.2 ms pulse duration), which was delivered by a Grass S44 Stimulator. The vasorelaxant responses induced by electrical field stimulation were analyzed after precontraction of arteries with a submaximal concentration of prostaglandin $F_{2\alpha}$ (10 μM), which yielded a stable level of contraction throughout the duration of the experiment. Segments were washed out for at least 60 min to avoid desensitization between subsequent electrical stimulation.

In some experiments, the electrical stimulation-induced relaxations were assessed in tissues precontracted with 5-hydroxytryptamine (5-HT, 3 μM), endothelin-1 (0.1 μM) or KCl (30, 50 and 70 mM).

The effect of 10 μM tetrodotoxin, 1 μM propranolol, 1 μM atropine, 30 μM indomethacin, 10 μM methylene blue, 100 μM N^G-nitro-L-arginine methyl ester (L-NAME), and 1 μM cimetidine (inhibitors of propagation of nerve impulses, cyclooxygenase, guanylate cyclase, nitric oxide synthase, muscarinic cholinergic receptors, β-adrenoceptors and histamine-2 receptors, respectively) on the relaxation elicited by electrical stimulation was determined by their addition to the organ bath 30 min before contracting the artery with prostaglandin $F_{2\alpha}$.

The relaxation induced by electrical stimulation in rat basilar arteries was also studied in the presence of the antioxidant sodium ascorbate (100 μM, 10 min preincubation) or superoxide dismutase (50 and 100 U/ml, 10 min preincubation) to analyze the possible participation of oxyradicals generated by electrical stimulation in the vasodilatory response.

Additional experiments were achieved to test the participation of K^+ channels in the relaxation induced by electrical stimulation using specific antagonists. Thus, glibenclamide (1 and 10 μM), apamin (0.1 and 1 μM) or charybdotoxin (0.1 and 0.2 μM) were administered to the organ bath 10 min in advance to block ATP-sensitive (K_{ATP}), low-conductance K_{Ca} (SK_{Ca}) and large-conductance K_{Ca} (BK_{Ca}) channels, respectively. The ability of the

K^+ channel activator cromakalim to cause vasorelaxation was determined in segments precontracted with 10 μM prostaglandin $F_{2\alpha}$. Likewise, the participation of BK_{Ca} and K_{ATP} in this response was examined by 10-min preincubation of segments with of 0.2 μM charybdotoxin or 10 μM glibenclamide.

It has been reported that an increment in the cytosolic free Ca^{2+} concentration may activate K_{Ca} channels (Kitazono et al., 1993; Nelson and Quayle, 1995). To examine if this mechanism is involved in the electrical stimulation-induced relaxation, experiments were performed in the presence of thapsigargin (0.01 μM and 0.1 μM, 10 min preincubation), an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, that increases the cytosolic free Ca^{2+} concentration (Marín et al., 1999). In addition, to assess if the effect of thapsigargin is due to activation of K_{Ca} channels, experiments were achieved by adding 0.2 μM charybdotoxin 10 min after 0.01 μM thapsigargin.

2.4. Data analysis and statistics

Contractile responses are expressed either as a percentage of the maximum level of tone (difference between the tone generated by 125 mM potassium-substituted physiologic salt solution and that produced by 0.2 mM papaverine), or as active wall tension (increase in vessel wall force above the resting level divided by twice the vessel segment length, in mN/mm). Relaxations are expressed in percentage of the induced precontraction. Results are given as mean ± S.E.M. Statistical analysis was done by means Student's *t*-test for paired or unpaired experiments, a *P* value less than 0.05 was considered significant.

2.5. Solutions and drugs

The composition of Krebs–Henseleit solution was as follows (mM): NaCl 115, $CaCl_2$ 2.5, KCl 4.6, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25 and glucose 11.1. The pharmacological agents used were: acetylcholine chloride, apamin, (±)-1,4-dihydro-2,6-dimethyl-5-nitro-4[2-(trifluoromethyl)phenyl]pyridine-3 carboxylic acid methyl ester (Bay K 8644), charybdotoxin, atropine sulphate, propranolol hydrochloride, cimetidine, cromakalim, endothelin-1, glibenclamide, 5-HT creatinine sulfate, indomethacin, methylene blue, L-NAME, papaverine hydrochloride, prostaglandin $F_{2\alpha}$, sodium ascorbate, superoxide dismutase (bovine liver, EC1.15.1.1) and thapsigargin purchased from Sigma (St. Louis, MO, USA); cromakalim and tetrodotoxin from Research Biochemicals International (Natick, USA).

Stocks solutions of drugs were made in bidistilled water, except cromakalim and glibenclamide in dimethylsulfoxide; prostaglandin $F_{2\alpha}$, tetrodotoxin, Bay K 8644 and thapsigargin in absolute ethanol; and indomethacin in bidistilled water containing 5% $NaHCO_3$. These solutions were kept at −20°C and aliquots of these solutions were diluted in Krebs–Henseleit solution just before use.

Drugs were added to the organ bath in volumes of 5 or 50 μl and the concentrations are expressed as final molar concentrations in the organ chamber.

3. Results

In intact segments of the rat basilar artery, the maximum relaxant response elicited by 10 μM acetylcholine was of 82 ± 3% ($n = 12$) over a previous and stable tone given with 10 μM prostaglandin $F_{2\alpha}$ of 1.46 ± 0.12 mN/mm (equivalent to 58 ± 4% of maximum level of tone), whereas in endothelium-denuded segments was of 8 ± 2% ($n = 10$, $P < 0.001$ vs. intact segments) over a similar tone. The maximum level of tone in arteries with endothelium was of 2.5 ± 0.12 mN/mm. The effective lumen diameter for artery segments with and without endothelium ranged between 255–319 μm, and 233–317 μm, respectively.

The electrical field stimulation of segments precontracted with 10 μM prostaglandin $F_{2\alpha}$ caused a rapid and potent relaxation when stimulated at single pulses of 0.2 ms. The initial relaxation to electrical stimulation was of 70 ± 6% ($n = 12$) of its prestimulation tension (Fig. 1); at five consecutive electrical stimuli, delivered every 60 min, the segments similarly relaxed; the respective values referred to their corresponding prestimulation tone were of 70 ± 9% ($n = 7$), 74 ± 8% ($n = 7$), 69 ± 5% ($n = 7$), 75 ± 3% ($n = 5$) and 72 ± 5% ($n = 5$). The relaxant responses to electrical stimulation were not modified by endothelium removal (Fig. 1). The contractile effect induced by 10 μM prostaglandin $F_{2\alpha}$ was unaltered by five successive additions to the bath at 6-min intervals. How-

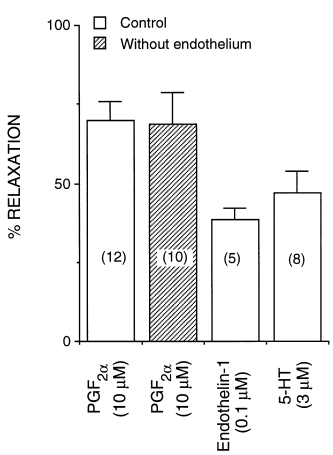

Fig. 1. Relaxation elicited by electrical stimulation (a single square-wave pulse, 0.2 ms, 200 mA) in rat basilar artery segments with or without endothelium and precontracted with different agents. Results (means ± S.E.M.) are expressed in percentage of contraction induced by each vasoconstrictor. Number of segments used is indicated in parentheses.

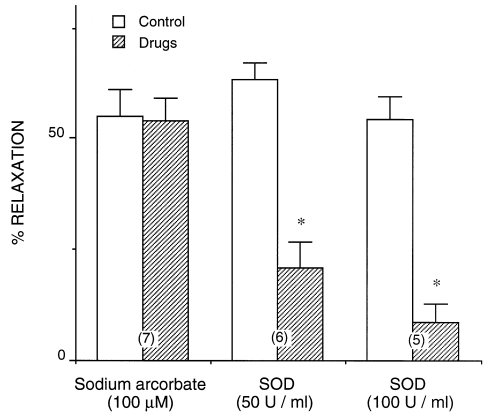

Fig. 2. Effect of sodium ascorbate and superoxide dismutase (SOD) on the relaxation induced by electrical stimulation (a single square-wave pulse, 0.2 ms, 200 mA) in rat basilar artery segments precontracted with 10 μM prostaglandin $F_{2\alpha}$. Results (means ± S.E.M.) are expressed in percentage of contraction induced by prostaglandin $F_{2\alpha}$. Number of paired experiments is indicated in parentheses. *: $P < 0.001$.

ever, electrical stimulation nearly suppressed the subsequent contraction induced by 10 μM prostaglandin $F_{2\alpha}$ added 6 min later (51 ± 5% before vs. 9 ± 2% after electrical stimulation, $n = 11$, respectively). The original response was recovered after a washout period of 60 min. When the single stimulus duration was increased (0.3 ms) a more pronounced vasodilation was observed, and the same occurred increasing the frequency to 0.1 Hz, although in this case the difference was not significant (77 ± 8% at 0.1 Hz, $n = 7$, vs. 70 ± 18% at 0.05 Hz, $n = 12$). For this, and to avoid an excessive production of chlorine gas and free radicals that could damage markedly and irreversibly the vascular contractile machinery (Hardebo et al., 1989), the smaller parameters initially indicated were usually used as they produced a clear relaxation.

In segments contracted with 0.1 μM endothelin-1 or with 3 μM 5-HT, which produced 72 ± 2% ($n = 5$) and 47 ± 6% ($n = 8$) maximum level of tone, respectively, electrical stimulation induced relaxations equivalent to 47 ± 7% ($n = 8$) and 39 ± 3% ($n = 5$) of their respective initial tone (Fig. 1). However, the contraction induced by 30, 50 and 70 mM KCl, that was of 56 ± 5% ($n = 14$), 68 ± 2% ($n = 15$), and 61 ± 4%, ($n = 13$), respectively, was not changed by electrical stimulation.

Treatment of segments with 10 μM tetrodotoxin, 1 μM propranolol, 1 μM atropine, 30 μM indomethacin, 10 μM methylene blue, 100 μM L-NAME or 1 μM cimetidine failed to alter the relaxation induced by electrical stimulation in prostaglandin $F_{2\alpha}$-contracted segments; relaxations obtained in the absence vs. presence of these respective drugs and expressed in % of the tone induced by 10 μM prostaglandin $F_{2\alpha}$ were: 69 ± 7 vs. 70 ± 6, $n = 9$; 61 ± 5 vs. 60 ± 3, $n = 5$; 64 ± 7 vs. 63 ± 6, $n = 5$; 80 ± 5 vs. 78 ± 5, $n = 8$; 61 ± 3 vs. 66 ± 5, $n = 7$; 77 ± 4 vs. 75 ± 5, $n = 5$; 65 ± 8 vs. 62 ± 8, $n = 6$. Only the three last drugs increased basal tone in 35 ± 8%, 20 ± 16% and 14 ± 3%, respectively, and the subsequent prostaglandin $F_{2\alpha}$-induced contraction was of 31 ± 5%, 47 ± 10% and 35 ± 5%, respectively.

Sodium ascorbate (100 μM) unaltered either the vasodilator response to electrical stimulation in prostaglandin $F_{2\alpha}$-contracted arteries (Fig. 2) or basal tone. However, superoxide dismutase (50 and 100 U/ml) reduced basal tone (11 ± 3%, $n = 6$, and 17 ± 5%, $n = 5$, respectively), as well as markedly decreased the vasodilator response to electrical stimulation (Fig. 2). Additional experiments were achieved with 50 U/ml superoxide dismutase to assess its capacity to antagonize the prolonged depressor effect that electrical stimulation exerted on prostaglandin $F_{2\alpha}$-induced contractions. In this condition, the response elicited, 6 min later, by prostaglandin $F_{2\alpha}$ continued significantly reduced (67 ± 8% before and 18 ± 4% after electrical stimulation, $n = 8$, $P < 0.001$). The original response was also recovered after a washout period of 60 min. In other experiments, 10 nM Bay K 8644 was added before the second period of electrical stimulation in segments precontracted with prostaglandin $F_{2\alpha}$. This Ca^{2+} agonist caused an increase in basal tone of 21 ± 4%, and the addition of prostaglandin $F_{2\alpha}$, 6 min later, recovered the contraction elicited by this agent (60 ± 4%) and the relaxation caused by electrical stimulation (47 ± 5%), ($n = 11$).

Glibenclamide produced a small increase of basal tone (1 μM: 2 ± 2%; 10 μM: 10 ± 5%, $n = 8$), and did not modify either the relaxation to electrical stimulation in segments precontracted with prostaglandin $F_{2\alpha}$ (Fig. 3) or

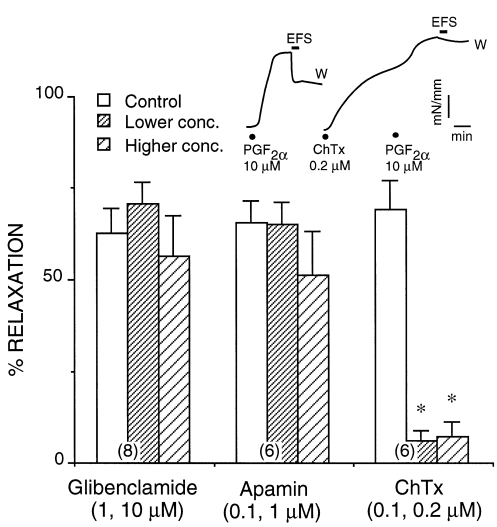

Fig. 3. Effect of two concentrations (conc.) of glibenclamide, apamin and charybdotoxin (ChTx) on the relaxation induced by electrical field stimulation (EFS, a single square-wave pulse, 0.2 ms, 200 mA) in rat basilar artery segments precontracted with 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Results (means ± S.E.M.) are expressed in percentage of contraction induced by prostaglandin $F_{2\alpha}$. A model showing the effect of ChTx on this relaxation is inserted in this figure, horizontal bars indicate the duration of electrical pulse. W, washout. Number of paired experiments is indicated in parentheses. *: $P < 0.001$.

the contraction elicited by this agent. Apamin (0.1 and 1 μM) did not change either electrical stimulation-induced relaxation (Fig. 3) or basal tone and the response induced by prostaglandin $F_{2\alpha}$. However, charybdotoxin caused a concentration-dependent contraction (0.1 μM: $42 \pm 6\%$; 0.2 μM: $59 \pm 7\%$, $n = 6$) and nearly abolished the relaxation elicited by electrical stimulation in these segments subsequently exposed to prostaglandin $F_{2\alpha}$ (Fig. 3). As charybdotoxin increased basal tone, we analyzed if this increased tone caused a physical limit for relaxant responses. For this, segments were precontracted with 3 μM 5-HT plus 10 μM prostaglandin $F_{2\alpha}$, that produced a contraction of $39 \pm 7\%$ plus $37 \pm 1\%$, $n = 8$, respectively, which was similar to that observed with 0.2 μM charybdotoxin plus 10 μM prostaglandin $F_{2\alpha}$ ($59 \pm 7\%$ plus $23 \pm 5\%$, $n = 6$, respectively). In these conditions, relaxations caused by electrical stimulation were not significantly different from those obtained in prostaglandin $F_{2\alpha}$-contracted arteries, suggesting that the increased tone caused by charybdotoxin is not a physical limit for relaxation. Thapsigargin (0.01 and 0.1 μM) increased basal tone (0.01 μM: $7 \pm 5\%$; 0.1 μM: $7 \pm 2\%$, $n = 9$) and the electrical stimulation-evoked relaxation in prostaglandin $F_{2\alpha}$-contracted segments (Fig. 4). This relaxation obtained in the presence of 0.01 μM thapsigargin was almost abolished by 0.2 μM charybdotoxin (Fig. 4).

The contraction induced by 10 μM prostaglandin $F_{2\alpha}$ in segments preincubated with 10 μM cromakalim was similar to that obtained in control situation. In addition, in prostaglandin $F_{2\alpha}$-contracted segments, cromakalim induced concentration-dependent relaxations that were reduced by 0.2 μM charybdotoxin, and specially by 10 μM glibenclamide (Fig. 5).

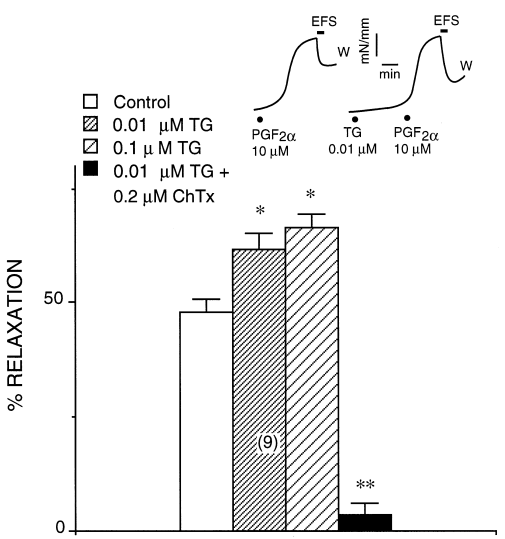

Fig. 4. Effect of thapsigargin (TG) on the relaxation induced electrical field stimulation (EFS, a single square-wave pulse, 0.2 ms, 200 mA) and its inhibition by charybdotoxin (ChTx) in rat basilar artery segments precontracted with 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Results (means ± S.E.M.) are expressed in percentage of contraction induced by prostaglandin $F_{2\alpha}$. A model showing the effect of TG on this relaxation is inserted in this figure, horizontal bars indicate the duration of electrical pulse. W, washout. Number of paired experiments is indicated in parentheses. *: $P < 0.01$, **: $P < 0.001$.

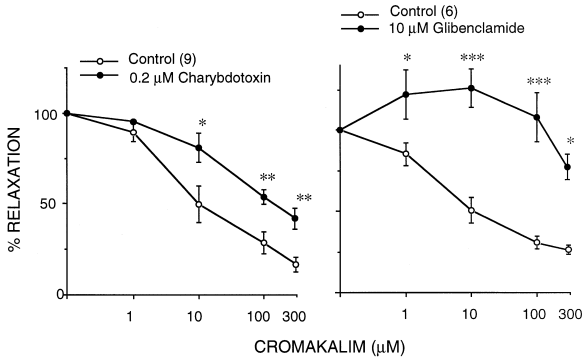

Fig. 5. Effect of charybdotoxin and glibenclamide on the relaxation elicited by cromakalim in rat basilar artery segments precontracted with 10 μM prostaglandin $F_{2\alpha}$. Results (means ± S.E.M.) are expressed in percentage of contraction induced by prostaglandin $F_{2\alpha}$. Number of paired experiments is indicated in parentheses. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.006$.

4. Discussion

The present results indicate that in precontracted rat basilar artery segments, electrical field stimulation, induced by a single pulse, caused a marked and sustained relaxation. This relaxation was observed when precontraction of segments was performed with prostaglandin $F_{2\alpha}$, 5-HT or endothelin-1. These findings suggest that electrical stimulation is able to induce relaxation independently of the agent used to induce the active tone. This relaxation was not modulated by endothelium, as similar relaxation was observed in endothelium-denuded segments. Similar results have been obtained in cerebral arteries (Hardebo et al., 1989) and in the rat tail artery (Geary et al., 1997). In contrast, an endothelium-dependent relaxation in cerebral arteries in response to electrical stimulation has been reported, mediated by endothelial nitric oxide (González and Estrada, 1991), or nitric oxide liberated from vasodilator nerves (Toda and Okamura, 1991).

The presence of histamine in cerebrovascular structures of rat and other animals has been reported (Holcslaw and Imhoff, 1978; Robinson-White and Beaven, 1982), as well as its ability to induce cerebrovascular relaxation, mainly mediated by histamine H_2 receptors (Edvinsson and Owman, 1975; Chang et al., 1988). The fact that the presence of the histamine H_2 receptor antagonist cimetidine did not alter the vasodilator response to electrical stimulation discards a possible histamine-dependent mechanism in the relaxation caused by electrical stimulation in this artery.

The occurrence of vasodilator innervations in rat cerebral arteries has been reported (Edvinsson et al., 1993; Tomimoto et al., 1993). However, the fact that the relax-

ation elicited by electrical stimulation was similar in the presence and in absence of the specific neurotoxin tetrodotoxin, that blocks the propagation of nerve impulses, suggests a non-neurogenic origin. Tetrodotoxin-resistant electrical stimulation-induced relaxation has been reported in pial arteries of different species (Hardebo et al., 1989). Atropine and propranolol, blockers of muscarinic cholinergic and β-adrenoceptors, respectively, did not alter this relaxation. Such results suggest that it is not mediated by either acetylcholine release from cholinergic nerves or noradrenaline release from adrenergic nerves which would activate β-adrenoceptors (Cohen et al., 1983).

The relaxation tetrodotoxin-resistant is not due to the stimulation parameters since the same (200 mA, 0.2 ms, González and Estrada, 1991) or higher (supramaximal intensity, 0.2 ms, Toda and Okamura, 1991; 300 mA, 0.3 ms, Chen and Lee, 1993) parameters produced tetrodotoxin-sensitive (neurogenic) relaxation in different cerebral arteries. The participation of free radicals on tetrodotoxin-insensitive vasorelaxation is unclear. Thus, in cerebral arteries, it is associated with free radicals formation and chlorine gas generation, specially at high parameters of stimulation (>0.3 ms, Hardebo et al., 1989). In contrast, in the rat tail artery, electrical stimulation (100–500 mA, 0.1 ms) induced relaxation tetrodotoxin-insensitive that was not mediated by free radicals (Geary et al., 1997). In our experimental conditions, the presence of the antioxidant sodium ascorbate did not modify the dilatory response to electrical stimulation. However, it was markedly reduced by the specific superoxide anion scavenger superoxide dismutase, indicating that is, in great part, mediated by superoxide anion generation. It is interesting to note that superoxide dismutase induced a relaxation of segments subjected to basal tone, probably due to the prevention of the nitric oxide metabolism formed in resting condition. Furthermore, the vasodilator response was not affected by indomethacin, which indicates that compounds derived from cyclooxygenase pathway are not involved. It is worthwhile to comment that after electrical stimulation, the contraction caused by prostaglandin $F_{2\alpha}$, in arteries preincubated or not with superoxide dismutase, remained reduced for 60 min. This fact suggests that the decreased response to this agent is not due to superoxide anion generation, but to other unknown mechanism. This transient alteration of prostaglandin $F_{2\alpha}$ response probably involves a desensitization or reversible damage of contractile machinery by this free radical, as reported for reactive oxygen species (Kontos et al., 1983), that decreases its sensitivity to Ca^{2+} (Iesaki et al., 1996). Indeed, the fact that 10 nM Bay K 8644, a Ca^{2+} channel agonist that facilitates the extracellular Ca^{2+} entry, specially in cerebral arteries (Salaices et al., 1985), abolished this depressor effect of electrical stimulation supports our assumption.

Once demonstrated, the participation of superoxide anions in the vasodilation induced by electrical stimulation, the mechanism implicated was analyzed. It is possible the involvement of peroxinitrite generated by the reaction of superoxide anion with nitric oxide (Marín and Rodríguez-Martínez, 1995), that may stimulate the soluble form of guanylate cyclase (Wu et al., 1994). The inability of nitric oxide synthase and guanylate cyclase inhibitors, L-NAME and methylene blue, respectively, to modify the vasorelaxant response to electrical stimulation indicates that these enzymes do not participate in such a response. It is worth noting that L-NAME and methylene blue increase basal tone, suggesting that this tone is modulated by a basal nitric oxide release.

The inability of electrical stimulation to induce relaxation in vessels precontracted with KCl suggests a participation of K^+ channels on the vasodilator response elicited by electrical stimulation. This suggests that the response is mediated by a hyperpolarizing mechanism, as in depolarized cells its expression is impossible. Furthermore, the increase of extracellular K^+ decreases the plasma membrane K^+ gradient, rending the K^+ channel mechanism ineffective, as reported (Khan et al., 1993). The activity of K^+ channels has an important role in the vascular tone regulation, specially in cerebral vessels, by hyperpolarizing smooth muscle cells (Wahl et al., 1994; Nelson and Quayle, 1995). Therefore, the activation of K^+ channels prevents that electrical stimulation may produce a higher depolarization of the smooth muscle cells, which would lead to a greater Ca^{2+} influx through voltage-operated Ca^{2+} channels, since K^+ channel activation closes these channels (Kitazono et al., 1993; Nelson and Quayle, 1995). For this, we analyzed the possible participation of K^+ channel blockers on vasodilator response to electrical stimulation in arteries precontracted with prostaglandin $F_{2\alpha}$.

K_{Ca} channels have been described in rat cerebral arteries (Wang and Mathers, 1993; Paterno et al., 1996), which are essentially BK_{Ca} and SK_{Ca} channels, selectively blocked by charybdotoxin and apamin, respectively; there is also K_{ATP} channels, inhibited by glibenclamide (Kitazono et al., 1993). It was observed that charybdotoxin significantly increased basal tone, indicating an important role of BK_{Ca} on the maintenance of vascular tone, as described (Brian et al., 1996); glibenclamide induced a slight increase, and remained unmodified with apamin, suggesting a less role of K^+ channels blocked by these agents in this tone regulation. In segments preincubated with glibenclamide or apamin, both the contraction caused by prostaglandin $F_{2\alpha}$ and the relaxation induced by electrical stimulation were unaltered. However, charybdotoxin produced a marked reduction of this relaxation. These results suggest that this response is caused by activation of BK_{Ca}.

The ability of superoxide anion to increase the cytosolic free Ca^{2+} concentration by facilitation of extracellular Ca^{2+} entry and Ca^{2+} release from intracellular stores has been reported (Hirosumi et al., 1988; Suzuki and Ford, 1992). This cytosolic Ca^{2+} increase in smooth muscle cells, probably caused by superoxide anion, may be the

mechanism involved in the activation of BK_{Ca} channels that produces vasodilation by hyperpolarization (Nelson et al., 1995). To confirm indirectly this assumption, thapsigargin was used, which is a known inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase that blocks Ca^{2+} uptake in this intracellular organelle, increasing cytosolic free Ca^{2+} concentration (Marín et al., 1999). Thapsigargin increased the relaxation induced by electrical stimulation, which was almost abolished by charybdotoxin. This result suggests that those mechanisms that increase cytosolic free Ca^{2+} levels, case of thapsigargin, potentiate the relaxation by activation of BK_{Ca} channels. Such a result indirectly supports the hypothesis that the relaxation elicited by electrical stimulation is mediated by activation of these channels due to intracellular Ca^{2+} increase caused by superoxide anions. To confirm the existence of such channels in the rat basilar artery, we analyze the ability of the K^+ channel opener cromakalim, that activates K_{ATP} channels (Ksoll et al., 1991) and also BK_{Ca} channels (Stockbridge et al., 1991; Gelband and McCullough, 1993), to induce relaxation in segments precontracted with prostaglandin $F_{2\alpha}$. In these conditions, cromakalim induced a potent vasorelaxation, which was significantly diminished by charybdotoxin and glibenclamide, confirming that cromakalim activate both kinds of K^+ channels, and that both are present in these arteries, as reported for cerebral vessels (Nelson et al., 1995). These results additionally suggest that K_{ATP} channels are present in these arteries, but they are not involved in the vasodilator response induced by electrical stimulation.

5. Conclusion

The present results show that single electrical pulses induce endothelium-independent and non-neurogenic relaxations in the rat basilar artery. These responses are mediated by generation of superoxide anions which, in turn, may induce hyperpolarization by activation of BK_{Ca} channels. The generation of these free radicals produces a reversible damage of contractile machinery. Although the precise mechanism involved in this process is unknown, the fact that it was increased by thapsigargin and almost abolished by charybdotoxin, suggests a process mediated by cytosolic free Ca^{2+} concentration increase that activates BK_{Ca} channels. K_{ATP} channels are present in this artery but does not participate in electrical field stimulation-induced relaxation. To our knowledge, this is the first time in which it is described the ability of electrical stimulation of short duration to produce vasodilation in cerebral vessels mediated by free radical production that generates a hyperpolarizing mechanism in which K^+ channels are involved. These radicals are normally generated in the cerebral tissue, and specially in ischemia-reperfusion processes (Marín and Rodríguez-Martínez, 1995), and hence the potential clinical interest of the present results.

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