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Effects of Calcium Channel Blockers on the Response to Endothelin-1, Bradykinin and Sodium Nitroprusside in Porcine Ciliary Arteries

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Calcium channel blockers are increasingly used in ophthalmology, for instance in patients with visual field defects caused by vasospasm. Endothelin is a new vasoactive peptide which also has been implicated in hypoperfusion of the ophthalmic circulation. This study investigated the effects of the calcium channel blockers on the response to endothelin-1, bradykinin and sodium nitroprusside in isolated porcine ciliary arteries (diameter 200–250 μm). Isolated porcine ciliary arteries were suspended in myograph systems filled with modified Krebs–Ringer solution (37°C; 95% O_2/5% CO_2) for isometric tension recording. Endothelin-1 (10^{-12}–10^{-7} M) induced potent concentration-dependent contractions of porcine ciliary arteries ($PD_{50} = 8{\cdot}3 \pm 0{\cdot}1$; $n = 7$). Lacidipine (10^{-5}–10^{-7} M) and nifedipine (10^{-5} M) significantly reduced the contractions and decreased the sensitivity to endothelin-1 as compared to control ($P < 0{\cdot}03$). On the other hand, endothelium-dependent relaxations to bradykinin ($10^{-10} - 10^{-6}$ M) and endothelium-independent relaxations to sodium nitroprusside (10^{-10}–10^{-4} M) remained unaffected by the calcium channel blocker. These findings demonstrate that in porcine ciliary arteries, the calcium channel blockers selectively inhibit endothelin-1-induced contractions, while leaving endothelium-dependent and endothelium-independent relaxations unaffected. This property of calcium channel blockers may contribute to the clinical efficacy of this class of drugs in patients with ocular vasospasms.

Key words: ciliary arteries; endothelin-1; lacidipine; serotonin; bradykinin; nitric oxide.

1. Introduction

The ocular circulation can be endangered by vasospastic syndromes. Indeed, patients with vasospasms have visual field defects (Kitazawa et al., 1989; Gasser and Flammer, 1987; Guthauser et al., 1988; Gaspar et al., 1994). Recently, calcium channel blockers which may prevent vasospasm-induced hypoperfusion of the optic nerve head have been studied in these patients (Flammer, 1993). Such vasospasm may involve the ciliary arteries and choroidal vessels or even optic nerve capillaries (Haefliger et al., 1994). However, the pathogenetic mechanism is not fully understood, being probably multifactorial. In vitro experiments have shown, that nitric oxide and endothelin-1 released by the endothelium are important regulators of vascular tone and in turn of ophthalmic blood flow (Yao et al., 1991; Haefliger et al., 1992; Meyer et al., 1993). In vasospastic patients treated with the calcium channel blocker nifedipine an improvement of the visual field defects was indeed observed in some cases (Gasser et al., 1988). In recent years, a large number of new molecules interfering with voltage-operated calcium channels have been developed. In contrast to the first generation of calcium antagonists such as nifedipine, verapamil and diltiazem, second generation calcium antagonists (which are most commonly of the dihydropyridine type) lack effects on cardiac contractility and conduction and often are much more potent as vasodilators than the first generation compounds. Indeed, lacidipine is a newly developed dihydropyridine-derivative with potent vascular effects (Mancia et al., 1993).

Dysfunction of these local regulatory systems could importantly contribute to alterations in ophthalmic circulation leading to vasospasm and in turn visual field defects. In patients with normal-tension glaucoma such a mechanism has been implicated and elevated plasma levels of endothelin-1 have been reported in these patients (Azuma, 1993; Kaiser et al., 1994).

Therefore, we investigated whether calcium antagonists used in clinical practice (i.e. nifedipine, lacidipine) would interfere with local vascular control mechanisms of isolated porcine ciliary arteries. In particular, we investigated the effects of calcium channel blockers on endothelin-1-induced vasoconstriction and on nitric-oxide-induced relaxations by bradykinin.

2. Materials and Methods

Preparation of Blood Vessels

Porcine eyes were obtained at an abattoir, 10 min after death of the animals and were transported in cold

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(4 °C) modified Krebs–Ringer bicarbonate solution of the following composition: NaCl 118·6 mM; KCl 4·7 mM; $CaCl_2$ 2·5 mM; $MgSO_4$ 1·2 mM; KH_2PO_4 1·2 mM; $NaHCO_3$ 25·7 mM; EDTA disodium salt dihydrate 0·026 mM and glucose 11·1 mM. Under a microscope, a short segment (7–8 mm) of the ciliary arteries (the two main branches of the common ophthalmic artery) was dissected free and cut into small rings (1·8–2 mm). During the preparation procedure, the tissues were constantly kept in cooled solution.

Experimental Equipment and Procedures

Experimental setup. After preparation, rings (diameters ranged between 200 and 250 μm) were immediately mounted in specially designed organ chambers for small vessels [myograph system; (Mulvany and Halpern 1977)]. Two stiff tungsten wires (diameters 30 and 50 μm) were carefully passed through the lumen and fastened to clamps attached to a force transducer (Showa Sokki LB-5, Rikadenki GmbH, Freiburg, Germany) and to a micromanipulator (Narishige, Tokyo, Japan) for adjustment of muscle length. The mounted rings were immersed in organ chambers filled with 12·5 ml of control solution (37°C; 95% O_2/5% CO_2, pH = 7·4) and equilibrated for 45 min.

The vessels were stretched stepwise as tension was increased and at each level of tension were exposed to 100 mM KCl. The optimal passive tension was defined as that tension at which the contraction to 100 mM KCl was maximal; this tension averaged 977 ± 60 mg in ciliary arteries ($n = 6$). In all subsequent experiments, arterial rings were stretched slowly in steps of 100 mg until optimal tension was reached. The active resting tone was defined as the difference between the optimal passive tension and the tension after maximal relaxation with bradykinin (10^{-6} M), the active resting tone averaged 174 ± 38 mg ($n = 6$).

Assessment of endothelial function. Before beginning the experiments, endothelial function was tested in each ring by adding bradykinin (10^{-6} M) after precontraction with serotonin (3×10^{-7} M). Full relaxation to bradykinin was taken as indicative of the presence of functioning endothelium.

Protocols. For each series of experiments, one ring of porcine ciliary artery from one eye of one animal was used; n therefore refers to the number of animals used in each series of experiments. After confirming endothelial function (see above) in each ring, the vessels were incubated with the calcium antagonist lacidipine (10^{-7}, 10^{-6}, 10^{-5} M respectively for 120 min), nifedipine (10^{-5} M for 120 min); other rings served for control experiments (75 μl absolute ethanol in 12·5 ml Krebs–Ringer solution). Then the vessels were contracted with increasing concentrations (cumulative dose–response curves) of endothelin-1 (10^{-12}–10^{-7} M).

To study the effect of the calcium antagonist on relaxations, vessels were precontracted with thromboxane analogue U-46619 (10^{-6} M) and studied in the presence or absence of lacidipine (10^{-7}–10^{-5} M, incubation 120 min). Then dose–response curves to bradykinin (10^{-10}–10^{-6} M) or sodium nitroprusside (SNP; 10^{-10}–10^{-4} M) were measured.

Drugs. Drugs were obtained from the following companies: bradykinin, sodium nitroprusside, serotonin and nifedipine from Sigma Chemicals Co (St. Louis, MO, U.S.A.), endothelin-1 from Novabiochem (Läufelfingen, Switzerland) and lacidipine from Boehringer Ingelheim (Basel, Switzerland). All drugs were dissolved in distilled water except the calcium antagonist lacidipine which was dissolved in 100% ethanol and endothelin-1 which was dissolved in Krebs solution, containing 0·05% bovine serum albumin. All concentrations are expressed as final molar concentrations in the organ chamber.

Statistical analysis. Contraction responses were expressed as a percentage of the maximal contraction to KCl, whereas relaxation responses were expressed as a percentage of the contraction induced by U-46619 (10^{-6} M). Maximal response (max), area under the curve (AUC) and concentrations evoking a particular percent of the maximal response to KCl (pD-values) were compared. The concentration causing 50% contraction (EC_{50} value) was expressed as negative log M concentration (pD_{50} value). Results are given as mean ± S.E.M. In all series of experiments, n equals the number of animals studied (one eye per animal). Unpaired Student's t-test was used for statistical analysis. A two-tailed P value smaller than 0·05 was considered to indicate a statistically significant difference.

3. Results

Endothelin-1-induced Contraction of Isolated Ciliary Arteries in the Presence and Absence of Calcium Channel Blocker

Endothelin-1 induced potent concentration-dependent contractions of porcine ciliary arteries with 140% of contraction elicited by 100 mmol KCl, with a pD_{50} value of 8·32 ± 0·12 (Fig. 1; $n = 7$). Preincubation of the vessels with lacidipine (10^{-7}–10^{-5} M) or with nifedipine (10^{-5} M) did not change significantly the vascular response compared to the control experiments. However, the preincubated substances significantly reduced the vascular response to endothelin-1 as compared to control rings incubated with ethanol (Fig. 1; $n = 5$–7). The area under the concentration-response curves (AUC) to endothelin-1 was significantly decreased after incubation with all concentrations of lacidipine as compared to control rings treated with ethanol ($P = 0·02$ for 10^{-7} M; $P = 0·03$ for 10^{-6} M and $P = 0·005$ for 10^{-5} M lacidipine). Similar results were obtained with nifedipine (10^{-5} M;

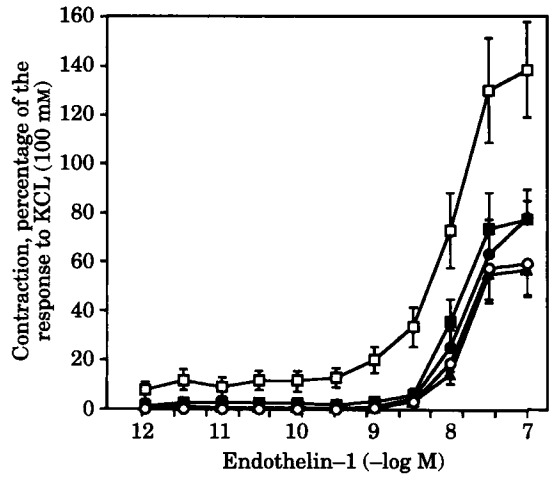

FIG. 1. Effect of calcium channel blockers (lacidipine 10^{-7}–10^{-5} M and nifedipine 10^{-5} M) on endothelin-1-induced vasoconstriction in porcine ciliary arteries: all concentrations of lacidipine and nifedipine inhibited the contraction to endothelin-1 as compared to control. □, control; ■, lacidipine (10^{-7} M); ●, lacidipine (10^{-6} M); ▲, lacidipine (10^{-5} M); ○, nifedipine (10^{-5} M).

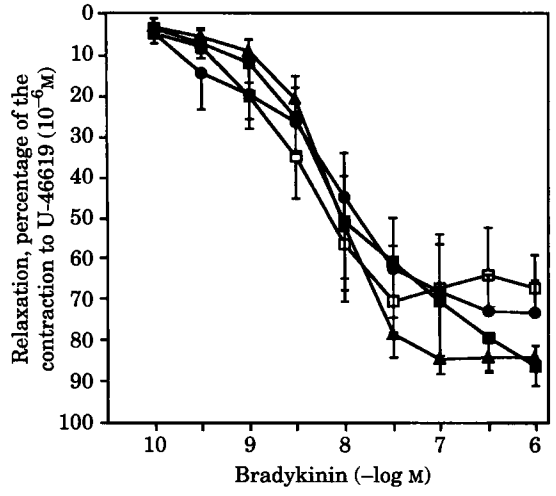

FIG. 2. Effect of calcium channel blocker (lacidipine 10^{-7}–10^{-5} M) on endothelium-dependent relaxations to bradykinin in porcine ciliary arteries: none of the concentrations of lacidipine affected the relaxations of ciliary arteries to bradykinin as compared to control. □, control; ●, lacidipine (10^{-7} M); ■, lacidipine (10^{-6} M); ▲, lacidipine (10^{-5} M). $n = 5$.

$P = 0{\cdot}006$ vs. control). The maximal effect of lacidipine (10^{-5} M) on endothelin-1-induced contractions was not different from that obtained by nifedipine (10^{-5} M; $P = 0{\cdot}896$). During the preincubation of the vessels with lacidipine (10^{-7}–10^{-5} M) or with nifedipine (10^{-5} M) compared to the control no significant vascular response occurred.

The sensitivity to endothelin-1 was also altered in the presence of the calcium antagonists. Indeed, the concentration–response curves were shifted to the right. The shift at pD_{50} was 4·7-fold ($P = 0{\cdot}01$) for lacidipine (10^{-7} M), 2·5-fold ($P = 0{\cdot}03$) for lacidipine (10^{-6} M), and 4·4-fold ($P = 0{\cdot}003$) for lacidipine (10^{-5} M). Similarly, nifedipine (10^{-5} M) shifted the

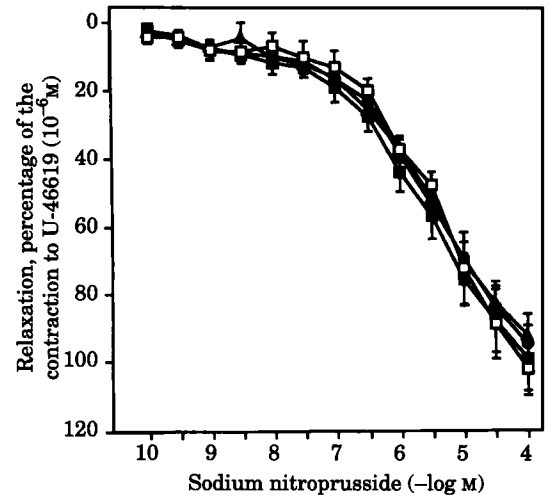

FIG. 3. Effect of calcium channel blocker (lacidipine 10^{-7}–10^{-5} M) on endothelium-independent relaxation to sodium nitroprusside: none of the concentrations of lacidipine affected the relaxation of ciliary artery to sodium nitroprusside as compared to the control. □, control; ●, lacidipine (10^{-7} M); ■, lacidipine (10^{-6} M); ▲, lacidipine (10^{-5} M). $n = 5$.

concentration–response curve 4·7-fold ($P = 0{\cdot}004$). The reduction of maximal response induced by the calcium antagonists averaged $44 \pm 11\%$ ($P = 0{\cdot}03$) for lacidipine (10^{-7} M), $44 \pm 21\%$ ($P = 0{\cdot}03$) for lacidipine (10^{-6} M), $59 \pm 19\%$ ($P = 0{\cdot}005$) for lacidipine (10^{-5} M) and $55 \pm 23\%$ ($P = 0{\cdot}01$) for nifedipine (10^{-5} M).

Endothelium-dependent Relaxations and Calcium Channel Blockers

In ciliary arteries, precontracted with the thromboxane analogue U-46619 (10^{-6} M), bradykinin (10^{-10}–10^{-6} M) evoked concentration–dependent relaxations. Preincubation of the arteries with lacidipine (10^{-7}–10^{-5} M) for 120 min, did not alter the relaxation of ciliary arteries to bradykinin (Fig. 2; area under the concentration–response curves n.s.; $n = 5$).

Endothelium-independent Relaxations and Calcium Channel Blockers

In ciliary arteries, precontracted with the thromboxane analogue U46619 (10^{-6} M) sodium nitroprusside (10^{-10}–10^{-4} M) induced concentration-dependent relaxations. Relaxations of ciliary arteries to sodium nitroprusside (10^{-10}–10^{-4} M) did not significantly differ in rings exposed to one of the three concentrations of lacidipine (10^{-7}–10^{-5} M) as compared to controls with ethanol (Fig. 3; area under the concentration–response curves n.s.; $n = 5$).

4. Discussion

The present study demonstrates that the calcium channel blockers such as lacidipine or nifedipine selectively inhibits contractions to endothelin-1 in

porcine ciliary arteries, while endothelium-dependent relaxations to bradykinin as well as endothelium-independent relaxations to sodium nitroprusside remained unaffected by the drug.

By acting on vascular smooth muscle, calcium channel blockers can lead to vasodilatation or relief from vasospasm. Two main classes of calcium channels exist: (1) voltage-operated channels, requiring membrane depolarisation for function, and (2) receptor-operated channels, requiring a specific ligand to bind to a receptor molecule linked to a channel. There are four types of voltage-operated channels: T-type, L-type, N-type and P-type (Bean, 1989; Pauwels et al., 1991; Catterall and Striessnig, 1992). Most calcium channel blockers, such as dihydropyridines like nifedipine and lacidipine, typically affect voltage-operated calcium channels and are targeted against L-type calcium channels. Lacidipine is a new 1,4-dihydropyridine that has vascular selectivity (Mancia et al., 1993). Nothing is known about its effects in ocular tissue. In healthy rabbits, however, another dihydropyridine, nifedipine increases choroidal blood flow (Miyoshi, 1984) as it does in vasospastic patients (Prünte and Flammer, 1989). Furthermore, nifedipine improved the visual field of young vasospastic patients in short- and long-term trials (Gasser and Flammer, 1990). Nifedipine did not reduce blood pressure in this group of patients who already had relatively low blood pressure before treatment. These studies suggest a clinical role for calcium antagonists in vasospastic patients.

In the present study, we examined the effects of the calcium channel blockers lacidipine and nifedipine on the vascular response to the potent vasoconstrictor endothelin-1. The physiological/pathophysiological role of endothelin is not yet defined, and plasma levels in healthy humans are low ($1{\cdot}11 \pm 0{\cdot}19$ pmol l^{-1}) (Sorensen, 1991). However, endothelin may be involved in the local regulation of vascular tone, and its vasoconstrictor effects may be of importance in the development of vasospasm in various vascular beds (Kurihara et al., 1989; Yang et al., 1990; Lüscher, 1991). Indeed, increased plasma levels of endothelin-1 have been demonstrated in patients with coronary vasospasm as well as with Raynaud's disease (Toyooka et al., 1991). Furthermore, in patients with normal-tension glaucoma [where ocular vasospasm is thought to play an important role; (Flammer et al., 1987)], plasma endothelin-1 levels are also higher than in those with high tension glaucoma (Azuma, 1993; Kaiser et al., 1994). If calcium antagonists are to be effective in these situations, they must interfere with the effects of endothelin-1 at the level of vascular smooth muscle. Indeed, in a randomized trial with healthy mountaineers with hypoxia-induced pulmonary hypertension at high altitude, nifedipine has been shown to lower pulmonary systolic pressure, while leaving the increase in plasma endothelin levels unchanged (Goerre et al., 1994).

Calcium antagonist, such as lacidipine or nifedipine block voltage-operated calcium channels. The fact that the drugs inhibit endothelin-1-induced vasoconstriction implies that endothelin-1 opens membrane voltage-operated calcium channels in the ciliary vascular smooth muscle cells, and that influx of extracellular calcium through these channels is in large part responsible for the maintainance of the contractions. Previous experiments by Nyborg et al. (1991) showed inhibitory effects of the calcium antagonist nitrendipine on endothelin-1-induced contractions in bovine retinal arteries. Similar observations were made for verapamil, a phenylalkylamine calcium channel blockers (Yu et al., 1992). The residual response to endothelin-1, in the presence of calcium antagonists, however, implies that other mechanisms of vasoconstriction are also involved. Indeed, in certain blood vessels, such as those obtained from the rat and rabbit (D'Orleans-Juste et al., 1989) and in the human internal mammary artery (Yang et al., 1990) calcium antagonist are ineffective. Endothelin-1 induced contractions resistant to calcium channel blockers are probably primarily mediated by release of intracellular calcium and activation of protein kinase C. The experiments of this study, therefore indicate that voltage-operated calcium channels are important for the endothelin induced contractions in porcine ciliary arteries. The fact that endothelin-induced contractions were more effectively suppressed by lacidipine or nifedipine at the lower concentration range may be related to the involvement of two endothelin-receptors (i.e. ET_A- and ET_B-receptors) as it has been demonstrated in other studies (Seo et al., 1994). Under our experimental conditions, both calcium antagonists were similarly effective; it remains possible that with prolonged incubation lacidipine exhibits more pronounced effects as it is known to be particularly potent as an inhibitor of calcium channels with prolonged periods of incubation.

In certain circulations, in particular in epicardial coronary arteries, calcium antagonists are able to facilitate the effects of nitric oxide on vascular smooth muscle (Küng et al., in press). Hence, we tested the effects of lacidipine on endothelium-dependent relaxations to bradykinin as well to endothelium-independent responses to sodium nitroprusside. Bradykinin releases nitric oxide from the vascular endothelium (Palmer et al., 1987). Sodium nitroprusside evokes vascular smooth muscle relaxation by directly increasing cGMP levels in vascular smooth muscle (Schultz et al., 1977; Rapoport et al., 1983). In porcine ciliary arteries, however, lacidipine did not influence the response to bradykinin or that to sodium nitroprusside, indicating that in this particular circulation, calcium channel blockage does not influence either nitric oxide production nor its effects at the level of vascular smooth muscle.

In conclusion, an L-type voltage-gated calcium channel blocker, which increases the optic nerve head

blood flow in experimental animals (Harino et al., 1992) and which clinically appears to protect against vasospasm-induced hypoperfusion in the ophthalmic circulation, markedly inhibits endothelin-1-induced contraction of porcine ciliary arteries, whilst leaving the production and action of nitric oxide unchanged in this part of the ocular circulation. Our findings suggest that blockade of voltage-operated Ca^{2+} channels might contribute to prevention or reversal of endothelin-induced vasospasm in ophthalmic circulation.

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