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Hydralazine-induced vasodilation involves opening of high conductance Ca^{2+}-activated K^+ channels

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Abstract

The purpose of this study was to investigate whether high conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) are mediating the vasodilator action of hydralazine. In isolated porcine coronary arteries, hydralazine (1–300 μM), like the K^+ channel opener levcromakalim, preferentially relaxed contractions induced by K^+ (20 mM) compared with K^+ (80 mM). In addition, concentration–relaxation curves for hydralazine ($pD_2 = 5.38 \pm 0.06$; $E_{max} = 85.9 \pm 3.6\%$) were shifted 10-fold to the right by the BK_{Ca} blockers tetraethylammonium (1 mM) and iberiotoxin (0.1 μM). In contrast, nimodipine (a Ca^{2+}-entry blocker), relaxed contractions induced by K^+ (20 mM) and K^+ (80 mM) equally and nimodipine-induced relaxations were neither antagonized by tetraethylammonium nor by iberiotoxin. In isolated perfused rat hearts, hydralazine (1 μM) increased coronary flow by 28.8 ± 2.7%. Iberiotoxin (0.1 μM) suppressed this response by 82% ($P < 0.05$). In conscious, chronically catheterized rats the hypotensive response to hydralazine (0.6 mg kg^{-1} min^{-1}) was significantly reduced by 41% during infusion of iberiotoxin (0.1 mg kg^{-1}). It is concluded, that opening of BK_{Ca} takes part in the mechanism whereby hydralazine produces vasodilation.

Keywords: Hydralazine; K^+ channel; High conductance Ca^{2+}-activated K^+ channel; Smooth muscle, vascular

1. Introduction

Hydralazine produces direct relaxation of smooth muscle in arteries and arterioles. It preferentially decreases vascular resistance in the coronary, cerebral and renal circulation with a smaller effect in skin and muscle. Although several theories have been put forward, the mechanism of action is still unknown (Gerber and Nies, 1990). It does not utilize established vasodilator mechanisms such as α-adrenoceptor antagonism, Ca^{2+} entry blockade or enhancement of cyclic AMP or cyclic GMP production. Membrane hyperpolarization due to activation of K^+ channels has recently been recognized as an important mode of action for several vasodilators (Edwards et al., 1992; Nielsen-Kudsk et al., 1996). This group of drugs include synthetic openers of ATP-sensitive K^+ channels (K_{ATP}) such as cromakalim, levcromakalim and pinacidil. However, also old vasodilators such as minoxidil and diazoxide have turned out to be K_{ATP} openers. Hydralazine has been reported to produce membrane hyperpolarization in rat caudal artery (Hermsmeyer et al., 1983) and in a previous study on isolated rabbit femoral arteries (Thirstrup and Nielsen-Kudsk, 1992) dihydralazine preferentially relaxed contractions induced by moderately raised K^+ (20 mM) compared with those induced by highly elevated K^+ (124 mM). This effect-profile is characteristic of drugs acting by K^+ channel opening, but glibenclamide, a specific blocker of K_{ATP}, failed to antagonize the vasorelaxation produced by dihydralazine. K^+ channels other than K_{ATP} are important in arterial smooth muscle. High conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) are activated by elevation of intracellular Ca^{2+} and by membrane depolarization and may serve as a negative feedback pathway to control the degree of membrane depolarization and vasoconstriction (Nelson and Quayle, 1995). BK_{Ca} are thought

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to play an important role in regulation of arterial tone (Brayden and Nelson, 1992).

Hydralazine has been used in the treatment of hypertension for decades. Additional to its antihypertensive action, the Vasodilator-Heart Failure Trials (VHeFT) have shown that hydralazine combined with isosorbide dinitrate improves exercise capacity and reduces mortality in patients with congestive heart failure (Cohn et al., 1986, 1991). This drug combination was of benefit even though isosorbide dinitrate in these trials was administered in a way (40 mg q.i.d.) in which development of nitrate tolerance should be expected. Intriguingly, recent studies in animals and humans indicate that hydralazine may prevent development of nitroglycerin tolerance (Bauer and Fung, 1991; Gogia et al., 1995). Consequently, determination of the mechanism of action of hydralazine might provide further understanding of the mechanism behind development of nitrate tolerance. In addition, recent data suggest that vasorelaxation in response to nitrovasodilators and nitric oxide (NO) involves activation of BK_{Ca} (Khan et al., 1993; Bolotina et al., 1994; Bialecki and Stinson Fisher, 1995).

Thus, the purpose of the present study was to examine whether BK_{Ca} are involved in the vasodilator action of hydralazine. The effects of BK_{Ca} modulation on hydralazine-induced vasorelaxation were investigated in isolated coronary arteries, isolated perfused rat hearts, the intact circulation of conscious rats and patch clamp experiments.

2. Methods

2.1. Isolated coronary arteries

Vascular ring segments (length 1.5 mm) were prepared from the distal epicardial part of the porcine left anterior descending coronary artery. The artery was excised immediately after slaughtering at a nearby abattoir. Each arterial segment was mounted in a precision myograph by insertion of two fine stainless steel pins into the vessel lumen. One of the pins was connected to a strain-gauge transducer for recording of isometric tension changes. The vessel myograph has been described in detail previously (Nielsen-Kudsk et al., 1986). Six myographs were used at the same time allowing six arterial preparations to be studied simultaneously. The transducer signals were amplified and displayed on an eight-channel Graphtec arraycorder (Graphtec, Japan). The vascular preparations were submerged in 5 ml tissue baths containing Krebs solution (composition in mM: NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15, glucose 5.5) at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2 (pH = 7.4). At the outset of each experiment the arterial rings were stretched to a resting tension of 2 g to optimize vasoconstrictor responses (maximal contractions induced by K^+-depolarization). Tissues were allowed to equilibrate for 1 h.

Vasodilators acting by opening of K^+ channels are expected preferentially to relax contractions induced by 20 mM K^+ as compared with 80 mM K^+. The vasorelaxant effect of hydralazine (1–300 μM), of the K_{ATP} opener levcromakalim (0.03–30 μM) and of the Ca^{2+} entry blocker nimodipine (0.0003–1 μM), which acts by a mechanism different from K^+ channel opening, was therefore examined in coronary arteries preconstricted either by 20 mM K^+ or 80 mM K^+. In another series of experiments, hydralazine (1–300 μM) and nimodipine (0.0003–1 μM) were further evaluated on coronary arteries preconstricted by prostaglandin $F_{2\alpha}$ (10 μM) either in the absence or presence of iberiotoxin (0.1 μM) or tetraethylammonium (1 mM). Iberiotoxin is a potent and highly specific blocker of BK_{Ca} (Garcia and Kaczorowski, 1992). Tetraethylammonium is a blocker of BK_{Ca} at concentrations ≤ 1 mM (Langton et al., 1991; Garcia and Kaczorowski, 1992). At higher concentrations it is a non-selective K^+ channel blocker. The BK_{Ca} blockers were added to the prostaglandin $F_{2\alpha}$ preconstricted arteries 20 min before addition of the vasodilators. Indomethacin (3 μM) was always present, when prostaglandin $F_{2\alpha}$ was used for preconstriction. The time intervals between concentration increments of the cumulatively added vasodilator drugs were for hydralazine 15 min, levcromakalim 10 min and for nimodipine 30 min.

2.2. Isolated perfused rat hearts

Male Wistar rats (250–280 g) were anaesthetized with pentobarbital (100 mg kg^{-1} i.p.) and then ventilated through a tracheal cannula using a rodent respirator (Ugo Basile, type 7025, Italy). After heparinization (1000 IE kg^{-1} i.a.), the chest was opened and the heart perfused in situ through a cannula inserted into the ascending thoracic aorta. The perfused heart was then excised and mounted in a Langendorff apparatus (type 830, Hugo Sachs Electronic, Germany). Hearts were electrically paced at a heart rate of 300 min^{-1} and the perfusion pressure was kept constant at 65 mm Hg. Coronary flow rate was measured continuously. The perfusion liquid was a modified Krebs–Henseleit solution (pH 7.4, 37°C, continuously aerated with a mixture of 95% O_2 and 5% CO_2) with the following composition in mM: NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15, glucose 5.5, pyruvate 2.0. Drugs were infused into the aortic perfusion line using adjustable precision syringe-pumps. The hearts were allowed to equilibrate for 30 min.

The effect of BK_{Ca} blockade on the coronary vasodilator effect of hydralazine was measured by the construction of concentration–response curves for hydralazine (0.01–100 μM) either alone or during infusion of tetraethylammonium (1 mM). The infusion rate for hydralazine was increased every 5 min. The tetraethylammonium-infusion was started 20 min prior to the infusion of hydralazine. Also the effect of hydralazine (1 μM, 5 min infusion)

either alone or during infusion of iberiotoxin (0.1 μM) was determined. The iberiotoxin-infusion was commenced 5 min before hydralazine.

2.3. Conscious rats

The in vivo effect of BK_{Ca} blockade on the vasodilator action of hydralazine was studied in conscious, unrestrained chronically catheterized male Wistar rats (280–330 g). After catheter implantation, rats were allowed to recover (6–8 days) until they appeared healthy and had regained their preoperative weight. Arterial blood pressure was continuously measured by a pressure transducer (Baxter, Holland) connected to an arterial catheter (medical-grade Tygon catheter) with its tip in the ascending aorta. Tracings were displayed on a Watanabe linearcorder (Watanabe Instruments, Japan). The animal model has previously been described in detail (Boesgaard et al., 1991). To evaluate the effect of BK_{Ca} blockade on the hypotensive response to hydralazine ($n = 6$), each rat received a 9 min continuous infusion of iberiotoxin (0.1 mg kg^{-1}) and iberiotoxin-solvent (0.9% NaCl). Hydralazine (0.6 mg kg^{-1} min^{-1}) was given during the last 4 min of each infusion period. The two infusions of iberiotoxin and iberiotoxin-solvent were given in a randomized order and were separated by a time interval of 24 h due to a long duration of the hypotensive effect of hydralazine. Similarly designed control experiments were performed to evaluate the reproducibility of the vasodilator response to hydralazine (iberiotoxin-solvent on both study days) and to estimate the effect of BK_{Ca} blockade on the response to nimodipine (nimodipine instead of hydralazine) ($n = 6$ in all groups). The doses of hydralazine and nimodipine were chosen to elicit a decrease in mean arterial pressure of approximately 30 mm Hg.

2.4. Patch-clamp studies

Bovine coronary artery smooth muscle cells were isolated from the circumflex and left anterior descending coronary artery. The medial layer was minced mechanically, and the explant cells were subcultured to passages 1–9. Cells were cultured on glass coverslips in Dulbecco's MEM medium (containing 2 mM glutamine and 10% fetal calf serum) and were used on day 1–5 after passage.

Whole-cell and single-channel currents were recorded by electrophysiological patch-clamp technique using a HEKA EPC-9 amplifier. Whole-cell currents were leak-subtracted and compensated for series resistance (80%). The ion composition of the extracellular solution was as follows (in mM): K^+ 4, Na^+ 140, Cl^- 150, Ca^{2+} 2, Mg^{2+} 1, Hepes 10. The pH was adjusted to 7.4 with HCl. The pipette solution always contained intracellular solution (in mM): K^+ 146, Cl^- 144, Ca^{2+} 1, Mg^{2+} 1, Hepes 10. EGTA was 2 mM, which gave a free internal Ca^{2+} concentration of 100 nM, pH was 7.2. The experiments were conducted at room temperature.

2.5. Data analysis and statistics

Vasorelaxant responses in isolated coronary arteries were expressed as percent relaxation relative to the level of preconstriction. Reversal of tone back to its baseline value was taken as 100% relaxation. In the myograph and Langendorff experiments, values of EC_{50} given as pD_2 values ($-\log EC_{50}$ values) and E_{max} were estimated by fitting the concentration–response curves to the three-parameter logistic equation (Hill-equation) using non-linear regression analysis. The significance of differences between pD_2 values was assessed by a two-way, unpaired Student's *t*-test. In the in vivo study, mean arterial blood pressure was estimated as diastolic pressure + (systolic pressure − diastolic pressure)/3 mm Hg. Comparison of mean arterial blood pressure values between the groups of animals was done by ANOVA (one way analysis of variance). Differences in vasodilator responses within the groups were assessed by Student's paired *t*-test. All data are presented

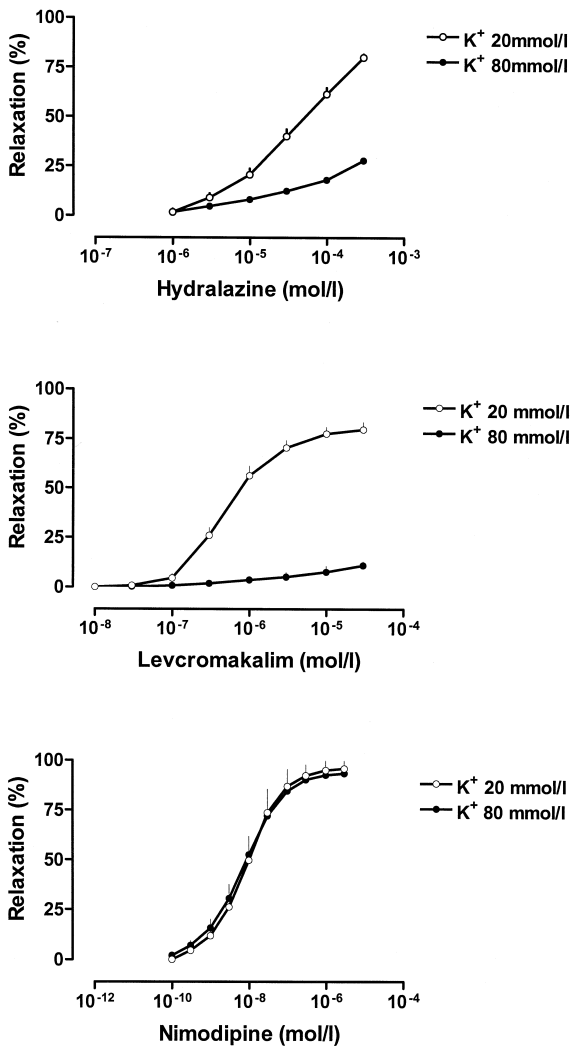

Fig. 1. Concentration–relaxation curves for hydralazine, levcromakalim and nimodipine in isolated porcine coronary arteries. The arteries were preconstricted either by 20 mM K^+ (○) or 80 mM K^+ (●).

as mean ± S.E.M. Statistical significance was assumed at $P < 0.05$.

2.6. Drugs

The following drugs were used: hydralazine hydrochloride (Sigma, USA), tetraethylammonium chloride (Sigma, USA), iberiotoxin (Sigma, USA), indomethacin (Sigma, USA), levcromakalim (SmithKline Beecham, UK), nimodipine (Bayer, Germany) and prostaglandin $F_{2\alpha}$ (Dinoprost trometamol, UpJohn, Belgium) and NS1619 (NeuroSearch, Denmark). Hydralazine (0.1 M), iberiotoxin (10 μM) and tetraethylammonium (0.1 M) was dissolved in 0.9% NaCl. The K^+-rich Krebs solutions used for K^+-depolarization in the myograph experiments were similar to the standard Krebs solution except that equimolar amounts of NaCl was replaced by KCl to make 20 and 80 mM K^+ solutions.

3. Results

3.1. Isolated coronary arteries

Depolarization by K^+-rich Krebs solutions produced stable contractions of 5.75 ± 0.33 g (K^+ 20 mM) and 6.97 ± 0.35 g (K^+ 80 mM), respectively ($n = 20$). Hydralazine selectively relaxed contractions induced by K^+ 20 mM compared with K^+ 80 mM. This effect-profile was shared by the K^+ channel opener levcromakalim, but not by the Ca^{2+} entry blocker nimodipine which relaxed the two types of contractions equally (Fig. 1).

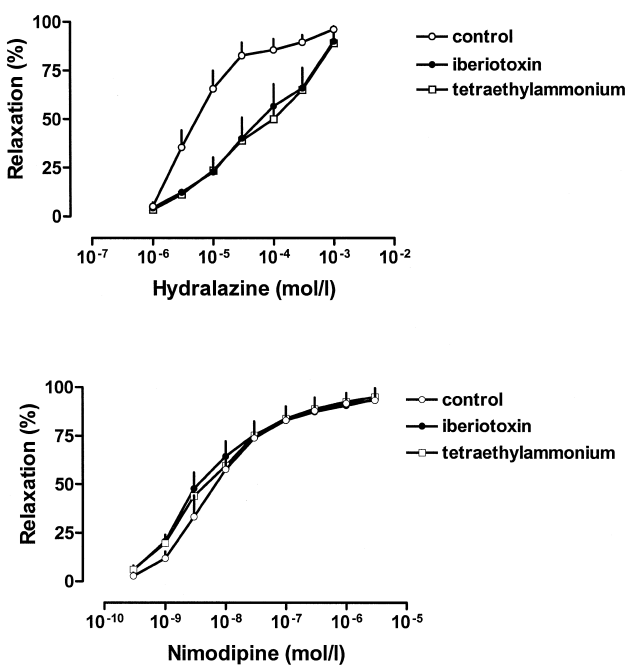

Fig. 2. Concentration–relaxation curves for hydralazine and nimodipine in isolated porcine coronary arteries preconstricted by prostaglandin $F_{2\alpha}$. The curves were obtained either in the absence (○) or presence of the BK_{Ca} channel blockers iberiotoxin (●) or tetraethylammonium (□).

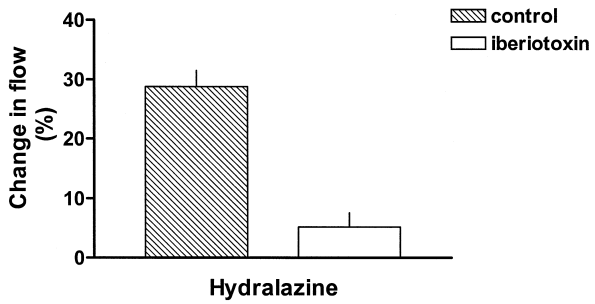

Fig. 3. Hydralazine-induced (1 μM) increase in coronary flow rate in isolated perfused rat hearts before (control) and after administration of iberiotoxin (0.1 μM)

Prostaglandin $F_{2\alpha}$ (10 μM) produced a stable contraction amounting to 6.96 ± 0.22 g ($n = 38$). Addition of iberiotoxin and tetraethylammonium caused a small additional contraction of 0.10 ± 0.01 and 0.09 ± 0.01 g, respectively. Hydralazine concentration-dependently relaxed coronary arteries preconstricted by prostaglandin $F_{2\alpha}$ ($pD_2 = 5.38 \pm 0.06$; $E_{max} = 85.9 \pm 3.6\%$). The vasorelaxation produced by hydralazine was significantly attenuated in the presence of both iberiotoxin ($pD_2 = 4.42 \pm 0.02$) and tetraethylammonium ($pD_2 = 4.31 \pm 0.06$). The pD_2 values indicate about 10-fold right-ward shift in the concentration–response curve for hydralazine in the presence of a BK_{Ca} blocker. In contrast, nimodipine which completely relaxed the prostaglandin $F_{2\alpha}$ preconstricted arteries ($pD_2 = 8.07 \pm 0.07$) was not antagonized by iberiotoxin ($pD_2 = 8.27 \pm 0.09$) and tetraethylammonium ($pD_2 = 8.21 \pm 0.06$), see Fig. 2 ($n = 5–8$ in all experiments).

3.2. Isolated perfused rat hearts

In isolated perfused rat hearts, hydralazine (0.01–100 μM) concentration-dependently increased coronary flow. The maximal increase in coronary flow rate was 54.3 ± 6.6% with a pD_2 value of 6.19 ± 0.28 ($n = 6$). During infusion of tetraethylammonium, the maximal effect of hydralazine was significantly suppressed ($E_{max} = 28.7 \pm 3.7\%$). Tetraethylammonium itself did not significantly change the basal coronary flow rate (10.3 ± 0.3 ml min^{-1}

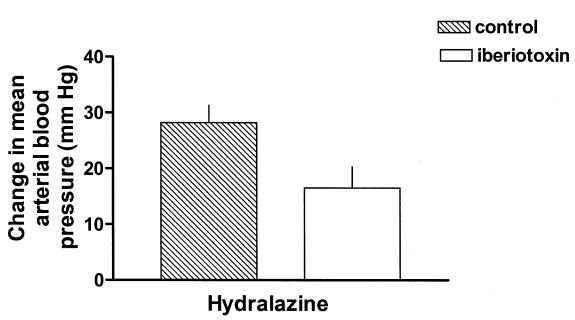

Fig. 4. Effect of control (iberiotoxin-solvent) and iberiotoxin infusion (0.1 mg kg^{-1} i.v. for 9 min) on the hypotensive response to hydralazine (0.6 mg kg^{-1} min^{-1} i.v. for 4 min) in conscious rats ($n = 6$). The two doses of hydralazine were separated by a time interval of 24 h. Iberiotoxin significantly attenuated the blood pressure lowering effect of hydralazine.

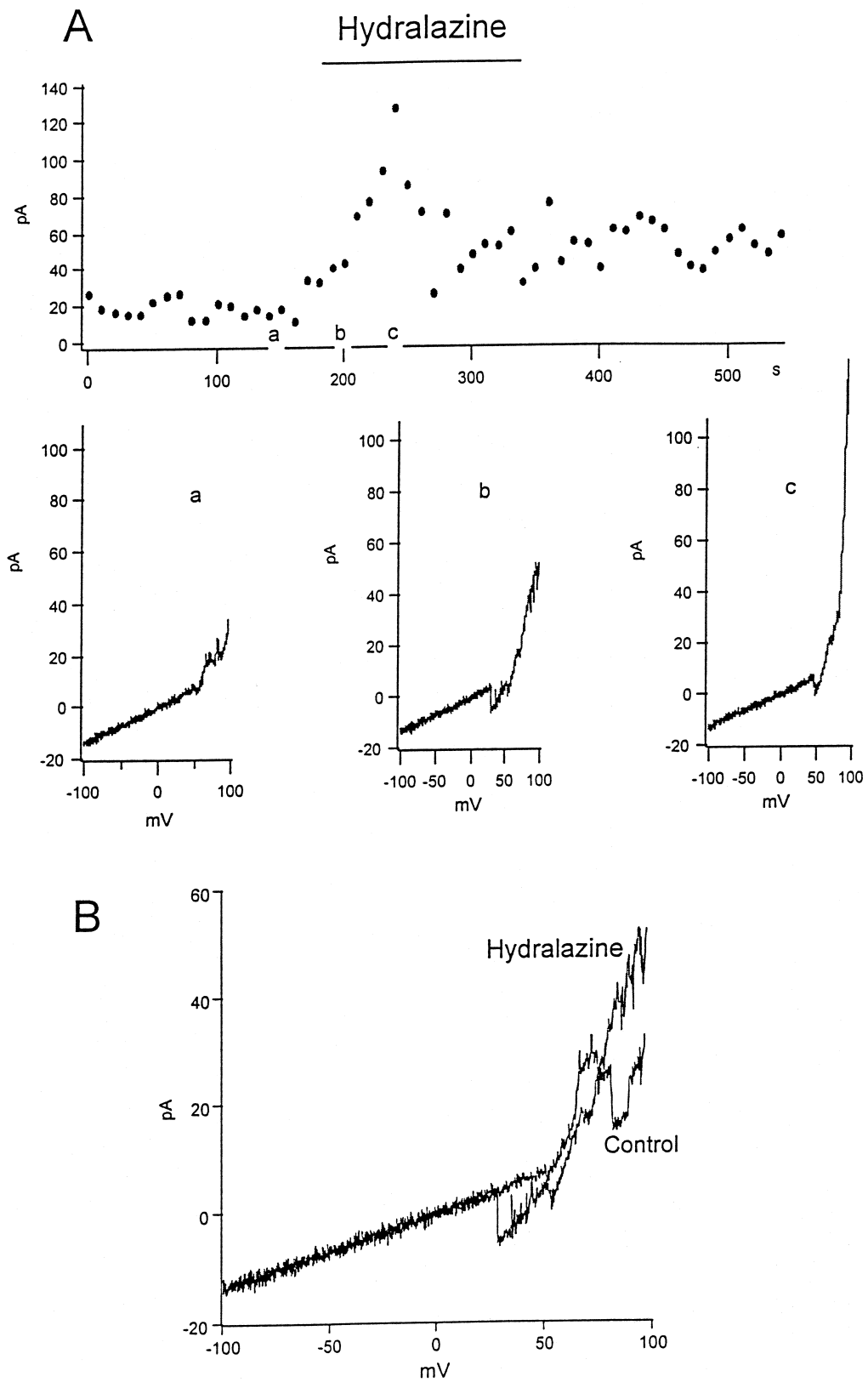

Fig. 5. Activation by hydralazine of single BK_{Ca} channels in a cell-attached patch from bovine coronary artery smooth muscle cells. (A) Hydralazine (300 mM) increased the mean outward current from 20 pA to about 70 pA (leak subtracted) as determined from the ramps at $V_m = 100$ mV. Each point represents the average of two recordings. The full current–voltage curves measured at the time points indicated by a, b and c are shown below. (B) Overlay of the current–voltage curves obtained immediately before (control) and 10 s after drug administration (hydralazine).

before vs. 9.8 ± 0.5 ml min^{-1} during tetraethylammonium).

Hydralazine (1 μM) increased coronary flow rate by $28.8 \pm 2.7\%$ ($n = 6$). During co-infusion of iberiotoxin (0.1 μM), the same dose of hydralazine only increased coronary flow rate by $5.2 \pm 2.4\%$, ($n = 6$, $P < 0.05$) (Fig. 3). Like tetraethylammonium, iberiotoxin (0.1 μM) did not significantly alter basal coronary flow rate (12.6 ± 1.0 ml min^{-1} before, 11.4 ± 1.0 ml min^{-1} during iberiotoxin).

3.3. Conscious rats

Mean arterial blood pressure (106 ± 1 mm Hg; $n = 18$) before the start of the experiments was similar in all groups and did not differ between the two study periods. Infusion of iberiotoxin and iberiotoxin-solvent had no effect on mean arterial blood pressure (before iberiotoxin: 102 ± 4 mm Hg, after iberiotoxin: 105 ± 4 mm Hg, $P > 0.05$). The hypotensive effect of hydralazine (0.6 mg kg^{-1} min^{-1}) was reduced by 41% after pretreatment with iberiotoxin as compared with iberiotoxin-solvent (from 29 ± 3 to 17 ± 2 mm Hg, $P < 0.05$) (Fig. 4). The response to hydralazine was reproducible on the two study days. Taken together, these data suggest, that the in vivo vasodilator response to hydralazine is significantly attenuated by administration of the BK_{Ca} blocker iberiotoxin. The hypotensive response to nimodipine was not influenced by BK_{Ca} blockade (34 ± 2 mm Hg vs. 31 ± 2 mm Hg, ($P > 0.05$)).

3.4. Patch-clamp study

3.4.1. Excised inside–out patches

Each patch contained 1–15 single channels of 250 pS conductance (symmetric 146 mM K^+). The channel activity was increased by depolarizing the membrane potential, by increasing the internal free Ca^{2+} concentration from 100 to 300 nM (Ewald et al., 1985). The channel activity was fully blocked by the BK_{Ca} channel blocker iberiotoxin (0.1 μM, $n = 24$) and activated by the BK_{Ca} channel opener NS1619 (10 μM, $n = 16$). Taken together these findings suggest, that the single channels are BK_{Ca} channels. Hydralazine (300 μM) did, however, not significantly modulate the channel activity during 4–12 min application ($n = 18$).

3.4.2. Cell-attached recordings

The activation curve of single BK_{Ca} channels was monitored each 10 second by applying a voltage ramp to the pipette ranging from −100 to +100 mV (duration: 250 ms). Hydralazine increased the channel activity as shown in Fig. 5. The mean outward BK_{Ca} current ($V_m = 100$ mV) was increased by 2.2 and by 2.8 fold, and the activation threshold was shifted 12 and 14 mV leftward by 100 and 300 mmol l^{-1} hydralazine, respectively ($n = 19$). The drug also hyperpolarized the smooth muscle cells by on average 4 mV as determined by the shift in reversal potential (Fig. 5) with no difference observed between 100 and 300 μmol l^{-1} concentration ($n = 19$).

3.4.3. Whole-cell recordings

In classical whole-cell recordings the dominating current was a tetraethylammonium and iberiotoxin-sensitive BK_{Ca} current with an activation threshold of about +50 mV (100 nM free Ca^{2+}) (Ewald et al., 1985). This current, which was measured by voltage ramps from −100 to +100 mV, was not significantly influenced by administration of hydralazine (300 mM) to the bath for periods up to 15 min ($n = 15$).

4. Discussion

The present ex vivo and in vivo experiments suggest, that opening of high conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) is involved in hydralazine-induced vasorelaxation.

In isolated coronary arteries, hydralazine preferentially relaxed contractions induced by 20 mM K^+ as compared with 80 mM K^+. The same effect profile was found for the K_{ATP} opener levcromakalim and this is a unique feature of drugs acting by K^+ channel opening. The vasorelaxant effect of the K^+ channel openers is abolished in media containing high concentration of K^+ (> 50 mmol l^{-1}), because high concentration of K^+ results in a shift in membrane potential towards the equilibrium potential for K^+ and this is the mechanism by which K^+ channel openers normally elicits vasodilation. The BK_{Ca} blockers tetraethylammonium and iberiotoxin antagonized the vasorelaxation produced by hydralazine, but did not influence the relaxation produced by nimodipine, a vasorelaxant acting by a mechanism different from K^+ channel opening. Thus, the antagonistic effect of tetraethylammonium and iberiotoxin against hydralazine cannot be explained by unspecific antagonism due to the small additional contraction induced by tetraethylammonium and iberiotoxin.

In the coronary circulation of isolated perfused rat hearts, BK_{Ca} blockade by tetraethylammonium and iberiotoxin did not significantly change basal coronary flow. However, these compounds produced a pronounced inhibition of the coronary vasodilator effect of hydralazine, suggesting that opening of BK_{Ca} take part in the mechanism whereby hydralazine increases coronary flow.

The in vivo experimental findings suggest, that activation of BK_{Ca} contributes to the vasodilator and hypotensive action of hydralazine in vivo. Iberiotoxin significantly attenuated the blood pressure lowering effect of hydralazine. This was a specific effect, because iberiotoxin per se neither affected blood pressure nor did it modify the vasodilator effect of nimodipine.

Direct electrophysiological support for hydralazine-mediated activation of BK_{Ca} was provided by the patch clamp experiments. In cell-attached recordings, hydralazine opened BK_{Ca} and hyperpolarized the cell membrane of coronary artery smooth muscle cells. In this mode of recording, the whole intracellular machinery of the cell is

intact. In excised membrane patches and in whole-cell recordings, hydralazine failed to modulate BK_{Ca} activity. With these recording modes, the integrity of the interior of the cell is lost and the findings therefore indicate, that hydralazine activates BK_{Ca} through a mechanism depending upon intact intracellular functions. One hypothesis of the action of hydralazine is, that it interferes with the Ca^{2+} handling of the sarcoplasmic reticulum inhibiting Ca^{2+} release and hereby mediating vasorelaxation (Gurney and Allam, 1995). In addition, phosphorylation and dephosphorylation of ion channel proteins are known mechanisms for modulation of ion channels including BK_{Ca} (Ewald et al., 1985) and might be involved in hydralazine mediated activation of BK_{Ca}.

Recent studies suggest, that hydralazine in animals and humans with heart failure may prevent the development of tolerance to organic nitrates like nitroglycerin (Bauer and Fung, 1991; Gogia et al., 1995). In a rabbit model of nitrate tolerance, hydralazine significantly decreased superoxide production (Münzel et al., 1996), which may be responsible for the development of nitrate tolerance. Similar effects have been shown with other hyperpolarizing agents like pinacidil, suggesting that membrane hyperpolarization due to K^+ channel activation might be the mechanism, whereby hydralazine affects tolerance development. Thus, modulation of vascular K^+ channels might represent a new concept for the prevention of nitrate tolerance.

In conclusion, the effects of hydralazine in isolated coronary arteries, in the isolated perfused heart and in the intact circulation of conscious rats were significantly influenced by inhibition of BK_{Ca}. The results suggest, that hydralazine-induced vasorelaxation involves opening of vascular BK_{Ca}.

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