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Short-term treatment with a beta-blocker with vasodilative capacities improves intrarenal endothelial function in experimental renal failure

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ABSTRACT

Aims: In patients with renal disease the cardiovascular risk is greatly increased, and endothelial dysfunction is assumed to play a pivotal role in this process. Therefore we compared treatment effects of a beta-blocker with additional vasodilatory capacities (nebivolol) and a beta-blocker lacking these actions (metoprolol) on intrarenal and coronary vascular function in a rat model of renal failure with hypertension.
Main methods: Renal failure was induced by 5/6-nephrectomy (Nx) and analyzed after 4 weeks in Wistar rats. Untreated Nx, Nx/nebivolol 6 mg/d (Nx-Nebi); Nx/metoprolol 60 mg/d (Nx-Meto) and sham-operated (Sham) animals were studied. Isolated small renal and coronary arteries were investigated for endothelium-dependent relaxation to acetylcholine (ACh) and for the contribution of the endothelial mediators NO and endothelium-derived hyperpolarizing factor (EDHF).
Key findings: Systolic blood pressure (SBP) was significantly increased in Nx, Nx-Nebi, and Nx-Meto (168 ± 5, 153 ± 3, and 162 ± 6 mmHg) compared to Sham (138 ± 3 mmHg, $p<0.05$, respectively). The increase in albuminuria of Nx (120-fold vs. Sham, $p<0.0001$) was almost (-85%) normalized by nebivolol compared to Sham ($p<0.05$), whereas metoprolol induced no significant effect. Renal arteries showed significantly increased Ach-relaxation in Nx and Nx-Nebi (Emax $86 \pm 4\%$ and $76 \pm 7\%$, $p<0.05$) due to an increase in EDHF-mediated dilation (Emax_EDHF $78 \pm 7\%$ and $73 \pm 6\%$) compared to Sham (Emax $54 \pm 4\%$ and Emax_EDHF $44 \pm 6\%$) and Nx-Meto (Emax $42 \pm 12\%$ and Emax_EDHF $18 \pm 5\%$). ACh-relaxation in coronary arteries was similar between groups but the contribution of NO (relative to EDHF) was strongly increased by nebivolol.
Significance: The present findings offer an explanation of the nephroprotective effect of intrarenal endothelial function in renal failure.

Introduction

Chronic renal disease is associated with an increased prevalence of cardiovascular complications (Ruilope et al. 2007). In patients with chronic renal disease the risk of dying of cardiovascular disease is even higher than the risk of progression to renal failure. The occurrence of systemic and coronary endothelial dysfunction associated with increased peripheral vascular resistance and coronary artery dysfunction in various stages of chronic renal disease has been proposed as an explanation for the increased rate of cardiovascular events (Gschwend et al. 2002a; Schiffrin et al. 2007). Moreover, specific intrarenal endothelial dysfunction may play an active role in the development and progression of renal damage itself (Gschwend et al. 2002b; Ochodnicky et al. 2006).

Impaired bioavailability of nitric oxide (NO) has been proposed to play a crucial role in endothelial dysfunction observed in chronic renal disease (Schiffrin et al. 2007). However, this condition is commonly associated with several other biochemical, metabolic and hemodynamic abnormalities such as hypertension that contribute to endothelial dysfunction and cardiovascular disease (Ruilope et al. 2007; Schiffrin et al. 2007). Thus, in addition to the blood pressure lowering effect the attenuation of endothelial dysfunction by antihypertensive medications represents an important additional goal to improve both renal and cardiovascular prognosis of patients with renal disease (Ruilope et al. 2007).

Beta-blockers belong to the standard repertoire of antihypertensive drugs and differ with respect to their mechanism of action, especially in terms of their β1-adrenoceptor selectivity and their

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vasoactive effects. A previously conducted study in hypertensive patients with type-2 diabetes mellitus demonstrated that beta-blocker carvedilol was superior to metoprolol in reducing albuminuria on top of blockade of the renin-angiotensin-system (Bakris et al. 2005). While this disparity on the regression of albuminuria could not be explained by blood pressure differences between the beta-blocker treatment groups, the authors concluded that the beneficial effect of carvedilol may be related to its ancillary action to reduce oxidative stress (Bakris et al. 2005).

In addition to carvedilol, also nebivolol could be of potential interest with regard to its nephroprotective potential (Hart and Bakris 2007), due to its additional vasoactive properties. Nebivolol is characterized by high ß1-adrenoceptor selectivity and shows stimulating effects on endothelial vasodilatory mediators such as NO (Pedersen and Cockcroft 2007). This notion is supported by a previous experimental long-term 6 month treatment study comparing nebivolol and atenolol in a renal mass reduction rat model of experimental renal failure (Pires et al. 2007). In this study the authors assessed endothelial function indirectly by measuring total vascular resistance changes in perfused hindlimbs of anesthetized animals. They demonstrated that the impairment of NO-dependent endothelial function after renal mass reduction was more efficiently improved by nebivolol than by atenolol. Although the effects on albuminuria or proteinuria were not analyzed in this investigation the authors observed a protective effect of nebivolol against renal structural damage, i.e. renal fibrosis. They concluded that these findings might be attributable to locally increased vasodilatation, decreased oxidative stress and/or increased NO bioavailability in response to nebivolol treatment (Pires et al. 2007).

Therefore, we set out to obtain further insights into the potential vascular mechanism contributing to the renoprotective effects of nebivolol in the 5/6-nephrectomy (Nx) rat model of renal failure. To this end we investigated the effects of short-term treatment with nebivolol for 4 weeks in isolated small renal (interlobar) arteries for endothelial function parameters and compared this to the effects in a different vascular bed, i.e. small coronary arteries. In addition, we differentiated the treatment effects on endothelium-dependent relaxation between NO and endothelium-derived hyperpolarizing factor (EDHF) dependent mechanisms. Furthermore, the effects on albuminuria, an important parameter reflecting dysfunction of the renal microcirculation in vivo (Bakris et al. 2005), were analyzed.

Materials and methods

Animals, surgery and in vivo measurements

Male Wistar rats (320–420 g) were housed under standard conditions at the animal facility of the Charité — Universitätsmedizin Berlin. The animals had free access to food and drinking water throughout the study. Surgery was performed under anesthesia with ketamin and xylazin. 5/6 nephrectomy was obtained by right nephrectomy and selective ligation of two to four extrarenal branches of the left renal artery leading to partial infarction of two-thirds of the left kidney. Postoperatively, rats received a subcutaneous injection of 1:10 diluted buprenorphin (Temgesic ®) for analgesia and were allowed to recover from surgery. The group of Nx rats was divided into subgroups ($n=8-9$, each) receiving either daily oral treatment with nebivolol (Nx-Nebi, 6 mg/d) or metoprolol (Nx-Meto, 60 mg/d), starting from day one after surgery. One subgroup of untreated Nx rats as well as Sham-operated (Sham) rats served as controls. Urinary albumin excretion was determined in all groups 4 weeks after surgery by placing the rats in metabolic cages for 24 h for urine collection and by using a sensitive and rat-specific enzyme-linked immunosorbent assay (ELISA) established in our laboratory (Kreutz et al. 2000). Furthermore, at this time systolic blood pressure (SBP) and heart rate in beats per minute (bpm) were measured in awake animals by means of the tail-cuff method (Schulz et al. 2002). At sacrifice 4 weeks after Nx, aorta, heart and kidney were excised and weighed. Blood was collected and serum samples were frozen (−20 °C). Laboratory investigations were performed as previously reported (Schulz et al. 2002). Animal experimentations were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

In vitro perfusion set-up

Overall, small renal (interlobar) and coronary (septal) arteries with an intraluminal diameter of 473 ± 11 µm ($n=43$) and 335 ± 9 µm ($n=27$), were prepared free and immediately transferred to an arteriograph system for pressurized arteries (Living System Instrumentation, Burlington, VT, USA) as reported (Gschwend et al. 2002a,b). Artery segments were cannulated at both ends on glass micropipettes, secured, and the lumen of the vessel was filled with Krebs solution through the micropipettes. Intraluminal pressure was set to 70 mmHg and held constant (blind sac) by a pressure servo system (Living System Instrumentation, Burlington, VT, USA). The vessel chamber was continuously recirculated with warmed (37 °C) Krebs solution with a pH of 7.4. The vessel chamber was transferred to the stage of an inverted light microscope with a video camera attached to a viewing tube. The video dimension analyzer (Living System Instrumentation, Burlington, VT, USA) was used to analyze the signal obtained from the video image and to continuously register lumen and vessel wall diameter.

Endothelium-dependent relaxation to ACh and endothelium-independent relaxation to Sodium Nitroprusside

Arteries were allowed to equilibrate for 1 h in regular Krebs solution before baseline diameter was determined. Then, arteries were pre-constricted with phenylephrine (renal arteries) or serotonin (coronary arteries) (3×10^{-7}–3×10^{-5} mol/L) by 62 ± 2% for subsequent relaxation studies. Preconstricted vessels were studied for endothelium-dependent relaxation by giving cumulative doses of acetylcholine (ACh; 10^{-8} mol/L–3×10^{-4} mol/L) to the recirculating bath. Afterwards, maximal endothelium-independent dilation was investigated using sodium nitroprusside (SNP, 10^{-3} mol/L).

Inhibition of the NO and EDHF pathway in ACh-induced relaxation

To determine EDHF-mediated relaxation and the relative contribution of nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF) to total ACh-relaxation, the response to ACh was additionally studied in presence of various inhibitors added to the bath 20 min prior to addition of ACh. N^{ω}-monomethyl-L-arginine (L-NMMA, 10^{-4} mol/L), given to the superfusion medium, was used to inhibit NO production, and a combination of charybdotoxin (10^{-7} mol/L) and apamin (10^{-7} mol/L), applied into the lumen of the artery as well as to the superfusion medium in presence of L-NMMA, was used to inhibit EDHF (Doughty et al. 1999; Jiang and Dusting 2001). To exclude any influence of vasoactive prostaglandins, indomethacin (10^{-5} mol/L) was present in the superfusion medium in all experiments to inhibit prostaglandin production. The exact nature of EDHF has not yet been established — meaning that specific inhibitors are not yet available. Nevertheless, the inhibition of calcium-dependent potassium channels with the combination of charybdotoxin and apamin has consistently been shown to inhibit the L-NMMA- and indomethacin-resistant relaxation and hyperpolarization which is believed to be mediated by EDHF (Jiang and Dusting 2001).

It is important to mention that the way in which the endothelial mediators are determined may be critical as they may not be independent but interact. In this context NO has been described to attenuate EDHF (release) (Bauersachs et al. 1996; Nishikawa et al. 2000) and thus, EDHF may be fully active only when NO is inhibited or