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Original Article

Influence of aldosterone *vs* endothelin receptor antagonism on renovascular function in liquorice-induced hypertension

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Abstract

Background. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) provides mineralocorticoid receptor specificity for aldosterone by metabolizing glucocorticoids to their receptor inactive 11-dehydro derivatives. Inhibition of 11β-HSD2 by liquorice-derived glycyrrhizic acid (GA) therefore results in sodium retention and hypertension. The present study investigated the effect of the aldosterone receptor antagonist spironolactone in comparison with the endothelin ET_A receptor antagonist darusentan on renovascular endothelial function in liquorice-induced hypertension.

Methods. GA, a recognized inhibitor of 11β-HSD2 was supplemented to the drinking water (3 g/l) of Wistar Kyoto rats over a period of 21 days. From day 8 to 21, spironolactone (5.8 ± 0.6 mg/kg/day), darusentan (45.2 ± 6.5 mg/kg/day), or placebo was added to chow ($n = 7$ per group). After the animals were killed, vascular function of isolated renal artery segments was assessed by isometric tension recording.

Results. Relaxation of pre-constricted renal artery segments in response to acetylcholine (10^{-10} to 10^{-5} mol/l) was impaired by GA as compared with controls ($12 \pm 4\%$ *vs* $98 \pm 5\%$ of norepinephrine 3×10^{-7} mol/l), whereas endothelium independent relaxations were unaffected. Endothelin receptor antagonism improved renovascular endothelium-dependent relaxation ($32 \pm 4\%$, $P < 0.05$ *vs* placebo) whereas endothelium-dependent relaxation was completely normalized by aldosterone receptor antagonism ($85 \pm 4\%$, $P < 0.01$ *vs* placebo).

Conclusions. In GA-induced hypertension, both aldosterone receptor antagonism and endothelin receptor antagonism normalize blood pressure and improve renovascular function and, thus, may represent a new therapeutic approach in cardiovascular disease associated with impaired 11β-HSD2 activity.

Keywords: endothelin-1; endothelium; glycyrrhizic acid; liquorice; nitric oxide

Introduction

The enzyme 11β-HSD2 is a member of the short-chain alcohol dehydrogenase superfamily, localized in the endoplasmatic reticulum with a cytoplasmatic-oriented catalytic domain. It confers mineralocorticoid receptor specificity by metabolizing glucocorticoids to their receptor inactive 11-dehydro derivatives [1]. Impaired renal conversion of cortisol to cortisone results in the congenital syndrome of apparent mineralocorticoid excess exerting sodium retention and severe hypertension [1,2] mediated in part through activation of both mineralocorticoid and glucocorticoid receptors [3]. As liquorice-derived glycyrrhizic acid (GA) is a well-known inhibitor of 11β-HSD2 [4], ingestion of GA results in 11β-HSD2-deficient hypertension. In addition to its role in the kidney, 11β-HSD2 has also been detected in cardiac fibroblasts, coronary artery vascular smooth muscle, and endothelial cells [5] where it is thought to regulate the response to steroid hormones released locally or systemically, and to modulate vascular tone and endothelial function. It is of note that reduced urinary excretion of steroid metabolites in patients with liquorice-induced hypertension as well as in untreated essential hypertension [6] has been observed, suggesting decreased activity of 11β-HSD2 in these patients.

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In patients with hypertension, endothelial dysfunction precedes the rise of blood pressure and predisposes to the development of structural vascular changes [7]. The endothelium releases vasoactive mediators such as nitric oxide (NO) and endothelin (ET)-1, both of which are importantly involved in the regulation of vascular tone [8] and structure [9]. Recently, endothelin receptor blockade has been demonstrated to effectively lower blood pressure in patients with mild to moderate hypertension [10]. In patients with heart failure, the aldosterone receptor antagonist spironolactone has recently been shown to increase NO bioavailability and vascular relaxations [11]. As several studies demonstrate beneficial effects of aldosterone receptor antagonism in heart failure [12,13], a renaissance for aldosterone receptor antagonism has begun. Furthermore, there is good body of evidence for a link between aldosterone and the endothelin system: ET-1 stimulates aldosterone secretion from adrenal cells [14] and has been demonstrated to regulate aldosterone production in patients with chronic congestive heart failure [15]. We recently demonstrated that in GA-induced hypertension, spironolactone is capable of normalizing vascular nitrate and ET-1 levels [16].

As renovascular haemodynamics play a pivotal role in the regulation of systemic blood pressure, the aim of the present study was to evaluate the effects of GA-induced hypertension on renovascular endothelial function. Moreover, we sought to compare the effect of aldosterone and endothelin receptor antagonism, respectively, on renovascular function in this model of hypertension.

Methods

Animals

Male Wistar Kyoto rats (mean weight 250 g, 13 weeks old) were obtained from RCC, Fuelinsdorf, Switzerland. GA (Sigma, Buchs, Switzerland) or vehicle was supplemented to the drinking water (3 g/l) over a period of 21 days. From day 8 to 21 rats were randomly assigned to treatment with either the orally available aldosterone receptor antagonist spironolactone (5.8 ± 0.6 mg/kg/day, Searle Monsanto, St Louis, MO, USA), the endothelin ET_A receptor antagonist darusentan (45.2 ± 6.5 mg/kg/day, Knoll AG, Ludwigshafen, Germany), respectively, or placebo administered with chow ($n = 7$ per group). Animals were maintained at 24°C, and kept at a 12 h light/dark cycle. Food and drug intake was monitored during the entire study and was adjusted for body weight. Dosages of spironolactone and darusentan were evaluated in preliminary studies and were chosen to achieve similar reduction in systolic blood pressure (SBP) among the treatment groups. SBP and heart rate (HR) were measured by the tail-cuff method using a pulse transducer (model LE 5000, Letica, Heerbrugg, Switzerland) [17]. An average of at least five independent measurements was taken. The study design and the experimental protocols were approved by the institutional animal care committee (Kommission für Tierversuche des Kantons Zürich, Switzerland).

Tissue harvesting

Animals were anaesthetized with pentobarbital (50 mg/kg i.p.) after 3 weeks treatment, and blood samples were collected through puncture of the right ventricle. Aorta and renal arteries were removed, dissected free from adherent tissue, and placed immediately into cold (4°C) modified Krebs–Ringer bicarbonate solution (mmol/l): NaCl 118.6, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.1, EDTA 0.026, and glucose 10.1. Under a microscope (Leica Wild M3C, Leica), renal arteries were cleaned of adherent tissue and cut into rings 3 mm long. Aortic tissue was snap frozen in liquid nitrogen for assessment of tissue ET-1 and nitrate levels.

Organ chamber experiments

Renal artery rings were suspended to fine tungsten stirrups (diameter 50 μM), placed in an organ bath filled with 25 ml Krebs solution and were connected to force transducers (UTC 2, Gould Statham, CA, USA) for isometric tension recording as described before [18]. After an equilibration period of 60 min, the rings were progressively stretched to their optimal passive tension (2.0 ± 0.2 g) (9). Rings were pre-constricted with norepinephrine (NE, ~70% of KCl 100 mmol/l) and relaxations to acetylcholine (Ach 10^{-10} to 10^{-5} mol/l) or sodium nitroprusside (SNP 10^{-11} to 10^{-5} mol/l) were obtained. Relaxations to Ach were assessed with and without pre-incubation of indomethacin (30 min, 10^{-7} mol/l) and in the presence or absence of the NO synthase (NOS) inhibitor nitro-L-arginine methylester (L-NAME) (pre-incubation for 30 min, 3×10^{-4} mol/l). In additional experiments, cumulative concentration-response curves to NE (10^{-10} to 10^{-4} mol/l) were obtained in quiescent preparations. Additional concentrations of drugs were added when contractions to the previous concentration were stable. All drugs used in the organ bath were obtained from Sigma Chemical Co. (Buchs, Switzerland) apart from ET-1 and big endothelin which were purchased from Novabiochem/Calbiochem AG (La Jolla, CA, USA). After experiments, vessel rings were blotted dry and weighed.

Calculations and statistical analysis

Relaxations to agonists in isolated arteries are given as per cent pre-contraction in rings pre-contracted with NE to ~70% of contraction induced by KCl (100 mmol/l). Contractions were expressed as percentage of 100 mmol/l KCl-induced contractions, which were obtained at the beginning of each experiment. Results are presented as mean ± SEM. In all experiments, n equals the number of rats per experiment. For statistical analysis, the sensitivity of the vessels to the drugs was expressed as the negative logarithm of the concentration that caused half-maximal relaxation or contraction (pD_2). Maximal relaxation (expressed as a percentage of pre-contraction) or contraction was determined for each individual concentration–response curve by non-linear regression analysis with the use of MatLab software. For comparison between two values, the unpaired Student's t test or the non-parametric Mann–Whitney test was used when appropriate. For multiple comparisons, results were analysed by ANOVA followed by Bonferroni's correction [19]. Pearson's correlation coefficients were calculated by linear regression. A value of $P < 0.05$ was considered significant.

Results

Body weight and standardized organ weight

Body weight did not differ between groups either before or after GA treatment nor during treatment with the aldosterone or the endothelin receptor antagonist, respectively (Table 1). This was paralleled by comparable kidney weight and heart weight among the treatment groups, as standardized for body weight: standardized heart weight was 3.7 ± 0.3 g/kg in controls *vs* 4.3 ± 0.3 g/kg on GA feeding *vs* 3.9 ± 0.4 and 4.3 ± 0.4 g/kg on treatment with darusentan and spironolactone, respectively.

SBP and HR

SBP increased on GA feeding and returned to baseline on either aldosterone receptor antagonism or endothelin receptor antagonism (Table 2) while HR was unchanged during the entire study and was comparable among the treatment groups (Table 3).

Contractions to NE and KCl

Concentration-dependent contractions to NE were unaffected by feeding with GA and aldosterone or endothelin receptor antagonism, respectively (Figure 1). Maximum contractions reached $119 \pm 4\%$ of KCl in controls *vs* $121 \pm 5\%$ of KCl on GA feeding *vs* $122 \pm 3\%$ of KCl and $125 \pm 2\%$ of KCl on treatment with darusentan and spironolactone, respectively. Contractile responses to 100 mM KCl did not differ between the groups (data not shown).

Endothelium-dependent relaxations

Native endothelium-dependent relaxations. Endothelium-dependent relaxations of renal artery segments to Ach were blunted by GA feeding (Figure 2A). After having reached a maximum relaxation ($62 \pm 8\%$) at a concentration of 3×10^{-7} mol/l Ach, relaxations in GA-fed animals were reduced at higher concentrations of Ach and finally reached $12 \pm 4\%$ at the highest Ach concentration, we evaluated (10^{-5} mol/l). In comparison, control animals exhibited $99 \pm 5\%$ relaxation at the highest Ach concentration ($P < 0.05$ *vs* GA feeding).

In contrast to endothelin receptor antagonism, which exhibited decreased relaxations at higher Ach concentrations ($32 \pm 4\%$ at 10^{-5} mol/l Ach; $P < 0.05$ *vs* controls), aldosterone receptor antagonism normalized endothelium-dependent renovascular endothelial

Table 1. Body weight (g) of Wistar Kyoto rats on feeding with GA and treatment with different regimens

Day	0	7	14	21
Control	269 ± 26	281 ± 34	296 ± 26	301 ± 19
GA + placebo	267 ± 17	271 ± 15	288 ± 19	293 ± 12
GA + darusentan	279 ± 22	291 ± 26	299 ± 28	317 ± 30
GA + spironolactone	258 ± 21	279 ± 12	289 ± 15	300 ± 19

Day 0 indicates body weight before treatment. Data are given as mean ± SEM of seven rats in each group. All differences n.s.

Table 2. SBP (mmHg) of Wistar Kyoto rats on feeding with GA and treatment with different regimens

Day	0	7	14	21
Control	145 ± 5	142 ± 9	145 ± 11	148 ± 10
GA + placebo	144 ± 7	184 ± 12*	186 ± 6*	192 ± 12*
GA + darusentan	145 ± 9	191 ± 15*	145 ± 7	146 ± 9
GA + spironolactone	146 ± 8	188 ± 12*	145 ± 7	142 ± 9

Day 0 indicates blood pressure before treatment. Data are given as mean ± SEM of seven rats in each group. *$P < 0.05$ *vs* control animals.

Table 3. HR (bpm) of Wistar Kyoto rats on feeding with GA and treatment with different regimens

Day	0	7	14	21
Control	382 ± 28	365 ± 28	334 ± 32	347 ± 32
GA + placebo	375 ± 28	367 ± 30	340 ± 24	335 ± 28
GA + darusentan	377 ± 32	341 ± 29	337 ± 26	342 ± 33
GA + spironolactone	387 ± 28	362 ± 29	374 ± 27	354 ± 26

Day 0 indicates HR before treatment. Data are given as mean ± SEM of seven rats in each group. All differences n.s.

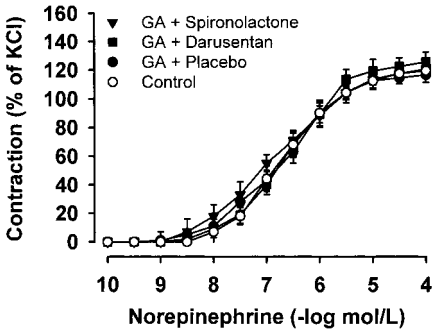

Fig. 1. Concentration-dependent contractions to NE in renal artery segments of Wistar Kyoto rats on feeding with GA and treatment with different regimens. Results are given as mean ± SEM ($n = 7$ per group).

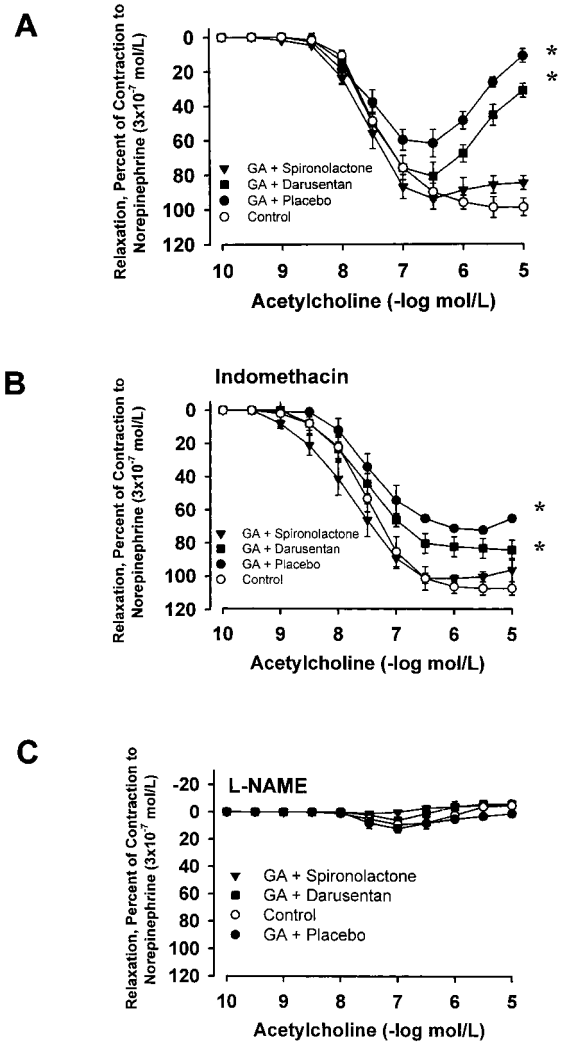

Fig. 2. Endothelium-dependent relaxations to Ach in renal artery segments of Wistar Kyoto rats on feeding with GA and treatment with different regimens. NO-mediated relaxations were obtained in quiescent preparations (A), after pre-incubation with indomethacin 10^{-7} mol/l (B) or L-NAME 10^{-4} mol/l (C), respectively. *$P < 0.05$ *vs* controls. Results are given as mean ± SEM ($n = 7$ per group).

function (85 ± 4% at 10^{-5} mol/l Ach; n.s. *vs* controls) (Figure 2A).

Endothelium-dependent relaxations in the presence of indomethacin. Reduced renovascular relaxation to higher concentrations of Ach was prevented by pre-incubation with indomethacin (65 ± 2% on GA feeding *vs* 84 ± 6% and 96 ± 7% for darusentan and spironolactone treatment, respectively) (Figure 2B) but differences between the treatment groups were still present ($P < 0.05$ *vs* controls for darusentan).

Endothelium-dependent relaxations in the presence of L-NAME. Relaxations to Ach were completely blocked by the NOS inhibitor L-NAME and no differences among the treatment groups were detectable (Figure 2C).

Endothelium-dependent relaxations in the presence of superoxide dismutase. Endothelium-dependent relaxations were unaffected by pre-incubation with superoxide dismutase (SOD): 100 ± 5% for controls *vs* 14 ± 7% on GA feeding and 29 ± 5% *vs* 78 ± 6% on treatment with darusentan and spironolactone, respectively (all n.s. in comparison to native endothelium-dependent relaxations).

Endothelium-independent relaxations. Endothelium-independent relaxations to SNP were comparable in all groups (Figure 3). Maximum relaxations reached 125 ± 3% in controls *vs* 119 ± 4% on GA feeding and 131 ± 6% on darusentan treatment *vs* 125 ± 4% on spironolactone treatment (all n.s. *vs* controls).

Discussion

These data show for the first time that in 11β-HSD2-deficient hypertension induced by ingestion of GA, aldosterone receptor antagonism is capable of normalizing endothelium-dependent vascular function in the renal artery. Endothelin receptor antagonism also improved renovascular endothelial function, but was not able to restore it completely.

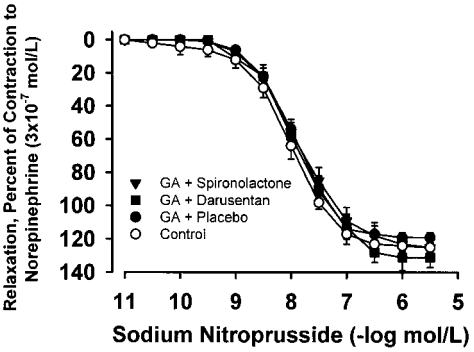

Fig. 3. Endothelium-independent relaxations to SNP in renal artery segments of Wistar Kyoto rats on feeding with GA and treatment with different regimens. Results are given as mean ± SEM ($n = 7$ per group).

Previously it has been shown that 11β-HSD2 activity, which regulates corticosterone (cortisol in man) access to its receptors and prevents inappropriate occupancy of type 1 mineralocorticoid receptors by glucocorticoids, is decreased in patients with liquorice-induced as well as salt-sensitive hypertension [6,20]. Interestingly, GA leads to competitive inhibition of 11β-HSD2 activity [21], which we recently could confirm for the present model of hypertension [22]. We provide here the first evidence that the administration of the 11β-HSD2 inhibitor GA leads to impairment of renovascular endothelium-dependent relaxation. As renovascular relaxations to Ach were completely blocked by L-NAME and were unaffected by SOD, these data are suggestive for an impairment of endothelial release of NO in GA-induced hypertension. It is of note that in higher concentrations of Ach, relaxations of renal arteries were reduced in a concentration-dependent manner. As this lack of appropriate vasodilatation can be prevented by pre-incubation with indomethacin, it is most likely mediated by vasoconstrictive prostanoids [23]. Taken together, hypertension induced by 11β-HSD2 inhibition may involve not only glucocorticoid and mineralocorticoid receptor-mediated modulation of renal function but also modulation of the renovascular NO system.

To further elucidate the impact of aldosterone and endothelin receptor antagonism on GA-induced endothelial dysfunction, animals were chronically treated with the aldosterone receptor antagonist spironolactone or the endothelin receptor antagonist darusentan, respectively, which both lead to a normalization of blood pressure.

Both compounds, spironolactone and darusentan, exhibited irreversible receptor blocking, which—in accordance with recent findings [16,24]—was represented by sustained improvement of endothelial function in isolated vessels *in vitro*.

The efficacy of spironolactone in the treatment of heart failure and hypertension in particular in states of sodium retention has been described [25]. In this study we provide the first evidence for complete reversibility of 11β-HSD2 inhibition-induced renovascular changes by chronic treatment with spironolactone. This is in line with the observation that spironolactone was able to normalize vascular nitrate and ET-1 levels in GA-induced hypertension [16]. It furthermore demonstrates a similar improvement of endothelial function in renal artery as compared with aorta [16]. As darusentan improved renovascular endothelium-dependent relaxation only in part, endothelin ET_A receptor-linked pathways may contribute, but not exclusively, to account for mediation of effects initiated by mineralocorticoid receptor activation. The normalization of blood pressure on darusentan treatment despite a certain degree of endothelial dysfunction suggests that other mechanisms than endothelial dysfunction—such as related to salt and water metabolism—may be considerable contributors to hypertension in this model.

Recent evidence indicates that elevated aldosterone levels play an important role in the development and progression of myocardial fibrosis and hypertrophy in congestive heart failure [13]. Further data support the hypothesis that sodium retention is not the primary mechanism of cortisol-induced hypertension [3]. These findings may be particularly relevant to the present study as the current data suggest that reduced activity of 11β-HSD2 could represent an important aldosterone-independent mechanism, through which inappropriate access of glucocorticoids to vascular receptors may influence vascular tone. The fact that aldosterone receptor antagonism has recently proven to decrease mortality in severe heart failure [13], emphasizes the importance of this therapeutic principle.

In conclusion, this study for the first time demonstrates that impairment of renovascular endothelium-dependent relaxation is a feature of hypertension induced by the 11β-HSD2 inhibitor GA, which can be restored by the aldosterone receptor antagonist spironolactone and, at least in part, by the endothelin ET_A receptor antagonist darusentan. Therefore, aldosterone and endothelin receptor antagonism may advance as beneficial treatment options in cardiovascular disease associated with reduced activity of 11β-HSD2.

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