Involvement Of $K_{Ca}3.1$ And $K_{Ca}2.3$ In EDH-type Relaxation In The Small Mesenteric Arteries Of Deoxycorticosterone Acetate-salt Hypertensive Rats Chronically Treated With FAAH Inhibitor URB597

Endothelial dysfunction associated with hypertension, is related with defects in the small ($K_{Ca}2.3$) and intermediate conductance ($K_{Ca}3.1$) calcium-activated potassium channels, which produce endothelium-dependent hyperpolarization (EDH)-mediated vasodilation (1). Fatty acid amide hydrolase (FAAH) inhibitors, including URB597, decreased blood pressure and caused vascular changes in hypertension (2). Thus, the aim of the study was to examine the involvement of $K_{Ca}2.3$ and $K_{Ca}3.1$ in EDH in isolated small mesenteric arteries (sMAs) of deoxycorticosterone acetate-salt (DOCA) hypertensive rats treated chronically with URB597. Uninephrectomized adult male Wistar Kyoto rats were divided into normotensive (UNX) and hypertensive (rats on a high salt diet and injected subcutaneously by DOCA; DOCA) animals. URB597 1 mg/kg or its vehicle [1 ml/kg; DMSO, Tween 80 and 0.9% NaCl (1:2:7)] were injected intraperitoneal twice daily for 14 days for both group of animals, UNX+URB, and DOCA+URB, respectively. Functional studies were performed in the isolated endothelium-intact sMAs using wire myograph (2). $K_{Ca}3.1$ expression was assessed by Western blots and by immunohistochemistry. Results are shown as means ± SEM of n animals as % of relaxation of phenylephrine(10 μM)-induced contraction. Statistical comparisons were made using one-way ANOVA followed by Bonferroni's post hoc test. $P<0.05$ was considered as statistically significant. EDH-type response was investigated as the acetylcholine (Ach; 0.001-30 μM)-evoked vasorelaxation in the presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME; inhibitor of nitric oxide synthase; 100 μM) and indomethacin (inhibitor of cyclooxygenase; 10 μM). The efficacy of EDH-type relaxation was similar in UNX and DOCA rats (Table 1), while affinity in DOCA was increased compare to UNX (6.5±0.1 vs. 6.1±0.1, P□0.05). Blockers of $K_{Ca}2.3$ and $K_{Ca}3.1$ [UCL1684 (0.1 μM) and TRAM34 (10 μM), respectively] given alone or in combination significantly attenuated and/or blunted EDH-mediated vasorelaxation in UNX and DOCA (Table 1). Concentration-dependent relaxation induced by NS309 ($K_{Ca}2.3$/$K_{Ca}3.1$ activator; 0.01-10 μM) was reduced in sMAs of DOCA compared with UNX and was sensitive to endothelium denudation and incubation with UCL1684 and TRAM34. URB597 treatment failed to affect all above EDH- and NS309-mediated responses in sMAs (Table 1). $K_{Ca}3.1$ expression in sMAs of DOCA-salt was reduced.

Table 1 Maximal relaxant effect (%) of EDH-like response induced by Ach and NS309

Group	n	UNX	n	UNX+URB	n	DOCA	n	DOCA+URB
EDH (Ach)	12	75.9±2.9	8	82.2±4.1	12	64.5±4.7	9	66.7±6.4
UCL1684	8	32.5±6.3$^{\Delta\Delta\Delta}$	6	41.1±7.5$^{\Delta\Delta\Delta}$	8	46.5 ± 3.5$^{\Delta}$	8	41.2 ±9.4$^{\Delta}$
TRAM34	8	8.6±5.4$^{\Delta\Delta\Delta}$	7	14.6±6.5$^{\Delta\Delta\Delta}$	8	35.1±3.4$^{\Delta\Delta\Delta;\#\#}$	5	38.0±5.2$^{\#\#;\Delta}$
UCL1684+TRAM34	5	9.5 ±3.2$^{\Delta\Delta\Delta}$	8	13.7±4.7$^{\Delta\Delta\Delta}$	5	17.6 ±4.9$^{\Delta\Delta\Delta}$	4	23.3±9.1$^{\Delta\Delta\Delta}$
EDH (NS309)	8	8□.8±4.6	5	74.4±1.2	8	68.5±6.3	4	63.2±5.8
UCL1684+T□AM□4	8	41.7±9.6$^{\Delta\Delta\Delta}$	4	55.4±9.9$^{\Delta\Delta\Delta}$	8	37.0±4.2$^{\Delta\Delta\Delta}$	4	37.4±7.0$^{\Delta}$
- endothelium	6	13.6±3.0$^{\Delta\Delta\Delta}$	4	21.4±5.5$^{\Delta\Delta}$	6	12.7±3.4$^{\Delta\Delta\Delta}$	3	22.7±3.8$^{\Delta\Delta}$

$^{\Delta}P<0.05$; $^{\Delta\Delta;\ \#\#}P<0.01$; $^{\Delta\Delta\Delta}P<0.001$ compared to the respective group ($^{\Delta}$EDH, $^{\#}$UNX)

In summary, the $K_{Ca}3.1$/$K_{Ca}2.3$-EDH-mediated relaxation was maintained in sMAs of hypertensive DOCA rats despite endothelium dysfunction and down-regulation of $K_{Ca}3.1$ and it was not affected by URB597. $K_{Ca}3.1$ channels played a key role in EDH-type dilator response of sMAs in normo- and hypertension.

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