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032P ADENOSINE $_{2A}$ RECEPTOR- INDUCED VASODILATION IN RAT PREGLOMERULAR MICROVESSELS IS MEDIATED BY EPOXYEICOSATRIENOIC ACIDS VIA cAMP/PROTEIN KINASE A PATHWAY

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The complex control mechanisms governing renal function are orchestrated within the preglomerular microvessels (PGMV); (Navar, 1998). Cytochrome P450-derived arachidonic acid metabolites (CYP-AA) and purines, e.g., adenosine, are mediators/modulators of renal function (Roman, 2002; Jackson *et al*., 2002). PGMV express high levels of CYP 4A and 2C / 2J families to generate vasoactive AA metabolites, e.g., a vasoconstrictor, 20-hydroxyeicosatetraenoic acid (20-HETE), and the vasodilators, epoxyeicosatrienoic acids (EETs). Adenosine, a potent endogenous physiological mediator, regulates renal vascular tone by stimulating G-protein coupled adenosine receptors (AR), A_1, and A_{2A} in PGMV. We therefore examined the effect of selective agonists of A_1 R, cyclohexyladenosine (CHA) and A_{2A} R, 2-p-(2-carboxyethyl)phenethylamino-5'-N-thylcarboxamidoadenosine (CGS 21680;CGS) on CYP-AA metabolism by PGMV.

PGMV from male Sprague Dawley rats (300g) were obtained using iron oxide, and incubated with CHA (10μM) or CGS (10μM) in Tyrodes solution, containing inhibitors of nitric oxide synthase (nitro-L-arginine methyl ester; 200μM) and cyclooxygenase (indomethacin; 10μM), for 15 min at 37^0C. After extraction and purification, CYP-AA were quantitated by GC-MS and expressed as ng/mg protein/15 min (Croft *et al*., 2000). Microdissected arcuate arteries were isolated, cannulated and pressurized to 80mmHg with Tyrodes solution. Arteries were preconstricted with phenylephrine (20nM) and internal diameter (i.d.) monitored by video microscopy.

CGS increased EET levels to 7.57± 1.53 ng from 1.06 ± 0.22 ng ($p<0.05$;n=6), without affecting HETE levels (10.8 ±0.69 vs. 11.02±0.74 ng). The levels of HETEs and EETs were unaffected by CHA. CGS-stimulated EET levels were abolished by an A_{2A} R antagonist, ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl) phenol (100μM) and a selective epoxygenase inhibitor, methylsulfonylpropargyloxyphenylhexanamide (MS-PPOH; 12μM). Arcuate arteries superfused with CGS (10μM) increased i.d. by 32±6 μm, responses that were prevented by MS-PPOH. Addition of 3nM 11,12-EET, 5,6-EET and 8,9-EET increased i.d. by 53±5 μm, 53±9 μm and 17±4 μm, respectively, whereas 14,15-EET was inactive. As CGS vasodilates by stimulating cAMP/protein kinase A (PKA) activity, we inhibited PKA activity with myristolated PKI (14-22) amide (5μM), and observed that the dilator responses to CGS and 11,12-EET were reduced by 88% and 95%, respectively. Further, inhibition of adenylyl cyclase (AC) activity with SQ22536 (10μM) reduced the responses to 11-12-EET by 63% and to CGS by 95%. As dilator responses to 8-bromo-cAMP (1mM) were unaffected by MSPPOH and did not stimulate EET release, we suggest that activation of A_{2A} R is coupled to EET release upstream of AC activation. Thus, in rat PGMV adenosine A_{2A} R-induced dilation is mediated by 11,12-/5,6-EET and by activation of AC/PKA.

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