Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue1abst012.pdf

Role Of Endothelial Mechanisms In Anandamide-Evoked Vasorelaxant Effects In Human Pulmonary Arteries

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Anandamide (AEA), an endocannabinoid derivative of arachidonic acid, is relevant mediator of regulation of vascular tone. AEA caused the endothelium-dependent vasorelaxation of the rat pulmonary artery via its cyclooxygenase-dependent products and O-1918-sensitive cannabinoid receptors (Baranowska-Kuczko et al. 2012). The influence of AEA on the human pulmonary arteries still remains unknown. Thus, **the aim** of the present study was to investigate the effects of AEA on pre-constricted isolated human pulmonary arteries.

Human isolated pulmonary arteries were obtained from 30 patients (24 men and 6 women, mean age 64.5±2.1 years) undergoing lobectomy or pneumonectomy during resection of lung carcinoma. Tissue donors had provided informed consent for the use of their vessels. Experiments were performed in tissue organ baths described by Kozłowska et al. (2008). Some tissue rings were pre-incubated with the following antagonists presented during the construction of the concentration-response curves for AEA: 30 min with O-1918 (10 µM); AM251, SR1445528 and RO1138452 (1 µM each) - antagonists of cannabinoid O-1918-sensitive, CB_1, CB_2 and prostacyclin IP receptors, respectively; URB597 (1 µM; fatty acid amide hydrolase inhibitor, FAAH); 45 min with capsazepine (1 µM; vanilloid TRPV1 receptor antagonist); indomethacin (10 µM; cyclooxygenase 1 and 2 inhibitor, COX); N^G-nitro-L-arginine methyl ester (300 µM; L-NAME; nitric oxide synthase inhibitor), iberiotoxin (0.1 µM; large conductance Ca^{2+}-activated K^+ channels inhibitor). One series was performed on endothelium-denuded arteries. Results are shown as mean ± s.e.m of n replicates. Statistical comparisons of the concentration response curves were made using an analysis of variance (ANOVA) followed by Dunnett's test or Student's t-test for unpaired data was used where appropriate. $P < 0.05$ was considered as statistically significant. The AEA-evoked vasorelaxation was expressed either as the % of relaxation of U-46619 (0.01 – 0.03 µM) or potassium chloride (KCl, 60 mM; only one group)-induced contraction. Maximal responses (R_{max}) and pEC_{25} were determined as described by Kozłowska et al. (2008).

AEA (0.1 - 100 µM) but not its vehicle (Tocrisolve; 0.001 – 1% v/v final concentration) relaxed concentration-dependently human pulmonary arteries pre-constricted with U-46619 (pEC_{25}=5.65±0.14; R_{max}=90.10±4.98, n=18). This effect was reduced by denudation of endothelium (pEC_{25}=4.18±0.11, P<0.001; R_{max}=22.93±10.70, P<0.001; n=7), in the presence of iberiotoxin (pEC_{25}=4.48±0.13, P<0.001; R_{max}=39.10±8.16, P<0.001; n=5), and was less potent under KCl-induced tone (R_{max}=24.88±12.38, n=5; P<0.001). AEA-evoked vasorelaxation was also decreased by indomethacin (pEC_{25}=4.53±0.32, P<0.01; R_{max}=28.20±10.45, P<0.001; n=7), L-NAME (pEC_{25}=4.24±0.13, P<0.001; R_{max}=35.68±10.39, P<0.001; n=4), URB597 (pEC_{25}=5.12±0.17, P<0.05; R_{max}=65.15±3.06, P<0.01; n=12) and RO1138452 (pEC_{25}=4.53±0.32, P<0.01; R_{max}=30.40±4.65, P<0.001; n=5). Further studies were carried out in the presence of URB597 in order to exclude the participation of AEA breakdown products. O-1918 reduced the AEA-evoked relaxation (pEC_{25}=4.43±0.13, P<0.05; R_{max}=37.85±3.45, P<0.05; n=7). Neither AM251 nor SR1445528 and capsazepine had changed the AEA-induced vasorelaxation.

These data demonstrated that AEA metabolites and prostacyclin receptors are involved in AEA-induced relaxation of endothelium-intact human pulmonary arteries. This mechanism may involve nitric oxide, potassium channels and O-1918-sensitive cannabinoid receptors.

Baranowska-Kuczko et al, Pharmacol Res 66:251, 2012

Kozłowska et al, Br J Pharmacol 155:1034, 2008

Supported by grants from the Medical University of Białystok (124-13505F) and The National Science Centre (2012/05/B/NZ7/03102).