Endothelium-Dependent Contractile Factor Release Is Related To Sphingolipid Alterations In Essential Hypertension

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We and others have previously shown that sphingolipids, a family of bioactive membrane lipids, can modulate endothelium-derived relaxing factor signalling. Since hypertension is associated with endothelial dysfunction, we investigated whether hypertension is associated with altered vascular sphingolipid biology and/or sphingolipid levels. In isolated carotid arteries from spontaneously hypertensive rats (SHR), pharmacological modulation of sphingolipid metabolism by means of exogenously applied sphingomyelinase (SMase, 0.1U/ml) or application of the sphingosine kinase inhibitor dimethylsphingosine (DMS, 10µM), induced marked transient contractions (2.1 ± 0.1 mN/mm and 1.4±0.4 mN/mm respectively; n=10). These contractions were virtually absent in vessels from normotensive Wistar Kyoto rats (WKY; 0.6±0.1mN/mm and 0.0±0.0 mN/mm respectively; n=9). Notably, the contractions were fully abolished by prior endothelium removal or by the cyclooxygenase inhibitor indomethacin (10µM; 0.0±0.0 mN/mm, n=7). To investigate which enzyme was mainly responsible for generating the COX substrate arachidonic acid, several phospholipase A₂ inhibitors were applied, of which the iPLA₂ inhibitor bromoenol lactone (25µM) almost completely inhibited SMase-induced contractions (0.2±0.1 mN/mm; n=6). In addition, the thromboxane synthase inhibitor ozagrel and the TP-receptor antagonist SQ29,548 concentration-dependently inhibited SMase-induced contraction, indicating that the contractions in SHR were mediated by thromboxane A₂. In accordance with aforementioned, DMS (10µM) augmented endothelin-1-induced contractions in isolated aorta of SHR (6.8±0.4 mN/mm and 5.1±0.4 mN/mm Eₘₐₓ in the presence or absence of DMS respectively; n=8-10, p<0.05), but not in WKY (10.2±0.3 mN/mm and 9.5±0.6 mN/mm). This DMS-induced augmentation was also endothelium-dependent and sensitive to COX inhibition and TP receptor antagonism, thus likely via augmented endothelin-1-induced thromboxane A₂ production.

Lipidomics analysis by mass spectrometry revealed significantly elevated levels of ceramide in arterial tissue and blood plasma of SHR (691±43pmol aorta, 645±25pmol plasma) compared to WKY (419±27pmol aorta, 513±19 pmol plasma) (n=6, p<0.05 SHR vs WKY). In order to investigate whether similar changes in sphingolipid biology may play a role in human hypertension we measured sphingolipid levels in plasma from normo- and hypertensive humans. We found that also plasma from humans with essential hypertension displayed elevated ceramide levels corresponding with the grade of hypertension severity (total ceramide: 183±11pmol normotension, 207±19pmol stage 1 hypertension, 243±23pmol stage 2+3 hypertension; n=18,12,19 respectively, p<0.05 normotension vs stage 2+3 hypertension).

In summary, we show that sphingolipids modulate the release and action of endothelium-derived contracting factors in hypertension and that (essential) hypertension is associated with marked alterations in sphingolipid biology and sphingolipid (plasma)levels.

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