Analysis of a new signaling pathway involved in thromboxane A_2 receptor-mediated vascular contraction

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Thromboxane A_2 (TXA_2), one of the eicosanoids, is an arachidonate product and exhibits its biological activity via G protein-coupled TXA_2 receptors, i.e., TP receptors. Although TXA_2 is well known to be a potent vasoconstrictor, the mechanism underlying the TP receptor-mediated vascular contraction remains to be fully elucidated. The present study was aimed to elucidate the mechanism, particularly focusing on lipid signaling.

U46619, a stable TXA_2 analog, induced vascular contraction consisting of two components in isolated ring preparations of rat aorta. The contraction induced by low-concentration U46619, i.e., at less than 100 nM, was highly sensitive to verapamil, a voltage-dependent Ca^{2+} channel (VDCC) blocker, whereas that induced by higher concentrations of U46619 was less sensitive to verapamil. Moreover, the former was abolished in a 0 Ca^{2+}/EGTA extracellular solution, whereas the latter was only partially inhibited under the condition.

We first investigated the mechanism underlying the extracellular Ca^{2+}-dependent contraction induced by low-concentration U46619. U46619 at 10 or 20 nM induced a sustained contraction of rat aorta, which was inhibited by ONO-RS-082, a non-selective phospholipase A_2 (PLA_2) inhibitor, and bromoenol lactone, a selective Ca^{2+}-independent PLA_2 ($iPLA_2$) inhibitor, but not by arachidonyl trifluoromethyl ketone, a selective cytosolic PLA_2 ($cPLA_2$) inhibitor. U46619 was also shown to stimulate arachidonate release in rat aorta, which was detected by liquid chromatography-Mass spectrometry. Moreover, the U46619-induced contraction remaining in the presence of verapamil was largely inhibited by ONO-RS-082 and 2-APB, a cation channel blocker.

Taken together with the results obtained in the present study, we propose the following mechanism: Low-concentration U46619 stimulates Ca^{2+} influx through VDCC and 2-APB-sensitive cation channels, which is primarily mediated by $iPLA_2$ signaling, thereby eliciting a sustained contraction of rat aorta.