We investigated how microsomal cytochrome P450 (Cyp450 MO) is regulated in cultured porcine aortic endothelial cells. The hypothesis that a Cyp450 MO-derived metabolite links Ca²⁺ store depletion and Ca²⁺ entry was studied further. Microsomal Cyp450 MO was monitored fluorometrically by dealkylation of 1-ethoxypyrene-3,6,8-tris-(dimethyl-sulphonamide;EPSA) in permeabilized cells or in subcellular compartments. Endothelial Ca²⁺ signalling was measured by a standard fura-2 technique, membrane potential was determined with the bis-(1,3-dibutylbarbituric acid) pentamethine oxonol (DiBAC4(5)) and tyrosine kinase was quantified by measuring the phosphorylation of a immobilized substrate with a horseradish peroxidase labelled phosphotyrosine specific antibody. Depletion of cellular Ca²⁺ pools with inositol 1,4,5-trisphosphate (IP3), thapsigargin or cyclopiazonic acid activated microsomal Cyp450 MO. Similar to direct Ca²⁺ store depletion, chelating of intramicrosomal Ca²⁺ with oxalate stimulated Cyp450 MO activity, while changing cytosolic free Ca²⁺ failed to influence Cyp450 MO activity. These data indicate that microsomal Cyp450 MO is activated by depletion of IP3-sensitive stores.

Besides the common cytochrome P450 inhibitors, econazole, proadifen and miconazole, thiopentone sodium and methohexitone inhibited Cyp450 MO in a concentration-dependent manner. The physiological substrate of Cyp450 MO, arachidonic acid, inhibited EPSA dealkylation. In contrast to most other cytochrome P450 inhibitors used in this study, thiopentone sodium did not directly interfere with Ca²⁺ entry pathways, membrane hyperpolarization due to K⁺ channel activation or tyrosine kinase activity. Inhibition of Cyp450 MO by thiopentone sodium diminished Ca²⁺/Mn²⁺ entry to Ca²⁺ store depletion by 43%, while it did not interfere with intracellular Ca²⁺ release by IP3 or thapsigargin. Cyp450 MO inhibition with thiopentone sodium diminished autacoid-induced membrane hyperpolarization. 7. Induction of Cyp450 MO with dexamethasone/clofibrate for 72 h yielded increases in thapsigargin-induced Cyp450 MO activity (by 35%), Ca²⁺/Mn²⁺ entry (by 105%) and membrane hyperpolarization (by 40%). The Cyp450 MO-derived compounds, 11,12 and 5,6-epoxyeicosatrienoic acids (EETs) yielded membrane hyperpolarization, insensitive to thiopentone sodium. 9. These data demonstrate that endothelial Cyp450 MO is activated by Ca²⁺ store depletion and Cyp450 MO produced compounds that hyperpolarize endothelial cells. 10. The data presented and our previous findings indicate that Cyp450 MO plays a crucial role in the regulation of store-operated Ca²⁺ influx. We propose that Cyp450 MO-derived EETs constitute a signal for Ca²⁺ entry activation and increase the driving force for Ca²⁺ entry by membrane hyperpolarization in porcine aortic endothelial cells.