● SERUM AND VASOCONSTRICTORS INCREASE PAI-1 PRODUCTION AND mRNA IN CULTURED RAT VASCULAR SMOOTH MUSCLE CELLS. G Sperti*, P Quax*, F Andreotti*, T Regan* , J Antoniw*, A Maseri * and C Kluft*, (intr. by C Brugnara) Cardiovascular Unit, Royal Postgraduate Medical School, London, U.K., and TNO Gaubius Institute , Leiden, NL.

Earlier reports have suggested that a plasminogen activator inhibitor (PAI) activity is associated with smooth muscle cells (SMC). We have tried to identify and characterize the PAI activity present in the supernatant of cultured rat aorta SMC and to ascertain whether it can be modulated by serum and vasoconstrictors such as serotonin (5HT) and angiotensin II (AII).

PAI-1 activity was assessed with a parabolic rate chromogenic assay in medium conditioned by confluent cultures challenged for 24hrs with the appropriate drug(s) after 48 hrs of serum deprivation. Northern blot hybridization was performed in stringent conditions with a human cDNA probe restricted to maximize the homology with the rat PAI-1 mRNA

5HT $1x10^{-4}$ M increased PAI-1 activity in the supernatant of SMC cultures from 18.0±3.1 IU / ml x mg protein to 35.8±4.9 IU / ml x mg protein representing an increase over control of 119±25 %. 5HT at a concentration of $1x10^{-5}$ M was equipotent when compared with the higher dose. The 5HT-induced increase in PAI activity was completely blocked by $3x10^{-7}$ M Ketanserin. A-II $1x10^{-7}$ M increased PAI-1 activity in the supernatant from 12.2±0 1 IU /ml x mg protein to 21.4±2.8 IU / ml x mg protein with an increase over control of 75±22 %. 10% Calf serum increased the PAI-1 activity in the supernatant from 9.9±1.4 IU / ml x mg protein to 26.4±6.3 IU / ml x mg protein (157±38% increase over control). Stimulation with 10% calf serum and 5HT $1x10^{-4}$ M increased total RNA ~2.5 and 1.5 times respectively. The signal / total RNA ratio remained nearly constant, indicating that serum and 5HT do not specifically increase PAI-1 mRNA, but several mRNA species including PAI-1.

In conclusion we have shown that PAI-1 activity and mRNA can be increased in VSM cultured cells by stimuli which are likely to be present in situations involving platelet deposition and the release of platelet-derived mediators (5HT and serum) and in certain forms of hypertension (A-II).