INTRACELLULAR REGULATION OF THE METABOLISM OF ARACHIDONIC ACID IN HUMAN PLATELETS. Thomas K. Bills, J. Bryan Smith and Melvin J. Silver. Thomas Jefferson University, Philadelphia, Pa., U.S.A.

The synthesis of prostaglandins and thromboxanes by human platelets is limited by the availability of the fatty acid precursor, arachidonic acid. Although large amounts of arachidonic acid are esterified to platelet phospholipids, only the free acid can be utilized by the oxygenation pathways of platelets. Since there are only trace amounts of free arachidonic acid in platelets, the enzymatic liberation of this fatty acid from platelet phospholipids can be considered the initial and rate limiting step of these oxygenation pathways. This process is catalyzed by a phospholipase A_2 whose role as the rate limiting enzyme makes it a prime target for the intracellular regulation of prostaglandin and thromboxane synthesis.

A second mechanism for the regulation of prostaglandin synthesis has been hypothesized. Several commonly occurring unsaturated fatty acids, e.g., oleic and linoleic acids, are capable of inhibiting prostaglandin cyclooxygenase. If these fatty acids are liberated from phospholipids along with arachidonic acid, they could limit the production of prostaglandins and thromboxanes. Thus, the types and amounts of fatty acids released from platelet phospholipids could regulate the amounts of prostaglandins and thromboxanes produced.

We have investigated these two types of intracellular regulation and have found that 1) intracellular cyclic nucleotides and divalent cations are involved in the regulation of the platelet phospholipase A_2 activity stimulated by thrombin and 2) this phospholipase A_2 activity catalyzes the specific release of arachidonic acid from phospholipids, thereby obviating the regulatory role of liberated oleic and linoleic acids.

THE PLATELET DENSE TUBULAR SYSTEM: ITS RELATIONSHIP TO PROSTAGLANDIN SYNTHESIS AND CALCIUM FLUX. Jonathan M. Gerrard and James G. White. University of Minnesota, Minneapolis, Minnesota, U.S.A.

Evidence that the dense tubular system (DTS) is the site of platelet prostaglandin synthesis derives from several observations. First platelet peroxidase is localized in the DTS. Aminotriazole which inhibits the platelet peroxidase, inhibits prostaglandin biosynthesis at the same concentration. Secondly, a similar peroxidase occurs in the secretory cells of the sheep vesicular gland (SVG) and other cells known to be involved in prostaglandin synthesis. Third, the DTS is smooth endoplasmic reticulum (SER) and SER, which is abundant in the prostaglandin synthesizing columnar cells of the SVG secretory units, is known to be involved in lipid synthesis and metabolism in other tissues. Evidence that calcium is stored in the DTS derives from ultrastructural studies showing that the DTS is analogous to the sarcotubules of skeletal muscle, and that the DTS has the capacity to bind divalent cations. Evidence that calcium flux and prostaglandin synthesis are closely linked comes from several observations which suggest 1) that movement of calcium ions to the site where arachidonic acid (AA) is released from the precursor phospholipids to be used for synthesis of prostaglandins, stimulates the AA release and 2) that synthesized prostaglandin G_2 , prostaglandin H_2 , and/or thromboxane A_2 initiate platelet contraction by moving calcium from the site of synthesis to the vicinity of the contractile actin and myosin. The results suggest that the DTS, prostaglandin and thromboxane synthesis, and calcium flux are integral parts of the system modulating platelet activation.

CYCLIC NUCLEOTIDES IN PLATELET FUNCTION. R.J. Haslam. Department of Pathology, McMaster University, Hamilton, Ontario, Canada.

Five lines of evidence indicate that cyclic AMP mediates the effects on platelets of inhibitory agonists, such as PGE_1 and adenosine. Thus, these compounds activate adenylate cyclase in platelet particulate fractions (1) and increase cyclic AMP levels in intact platelets (2), while their effects are potentiated by inhibitors of cyclic AMP phosphodiesterase (3), are blocked by inhibitors of adenylate cyclase (4) and are mimicked by analogues of cyclic AMP (5). Apparent discrepancies between platelet cyclic AMP levels and the inhibition of aggregation can be explained by the ability of some aggregating agents to prevent increases in cyclic AMP and by postulating a delay before the effect of a change in platelet cyclic AMP level is expressed. A mechanism of action for cyclic AMP is suggested by observations that it causes phosphorylation of platelet membrane proteins and increases the uptake of Ca^{2+} ions by platelet membrane fractions. Studies on the effects of aggregating agents on cyclic AMP levels in resting platelets have given variable results but recent work indicating that compounds that can inhibit adenylate cyclase in intact platelets neither cause nor reproducibly potentiate aggregation or release suggests that cyclic AMP plays no role in the responses of platelets to aggregating agents, unless the platelet cyclic AMP level is elevated above resting values. Studies on platelet cyclic GMP levels have shown that increases usually occur in association with both platelet aggregation and the release reaction. While these findings are consistent with some role for cyclic GMP in these processes, conditions have been found in which some aggregation and release can occur without increases in cyclic GMP. In general, the available evidence suggests that Ca^{2+} ions are likely to be more important than cyclic GMP in mediating platelet responses.