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SUBCELLULAR LOCALIZATION OF HUMAN PLATELET LIPOXYGENASE ACTIVITY: COMPARISON BETWEEN NORMAL AND LIPOXYGENASE-DEFICIENT PLATELETS.

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Lipoxygenase activities were estimated in platelet subcellular fractions as well as in intact platelets obtained from normal subjects and patients with deficient platelet lipoxygenase activities (< mean - 2SD of normal activities). From a washed platelet suspension (intact platelets), subcellular fractions including 12,000 x g supernatant of sonicated platelets (F-I), 105,000 x g supernatant (cytosol, F-II) and sediment (microsomal fraction, F-III) of F-I were prepared by differential centrifugation at 4°C. The enzyme activity was studied by the determination of 12-hydroxyeicosatetraenoic acid (HETE; ng) produced by the reaction of 100 μM arachidonic acid with 10^8 platelets or the subcellular fraction derived from them at pH 7.4 for 5 min at 37°C in the presence or absence of 2.5 mM $CaCl_2$ and/or 2 mM ATP by the use of reversed-phase high-performance liquid chromatography. In experiments with subcellular fractions, reduced glutathione was added to the reaction mixture. In normal subjects, HETE production by intact platelets, F-I, F-II and F-III was 1,162.4 ± 203.3, 1,029.7 ± 403.8, 368.8 ± 175.8 and 194.4 ± 73.4 (M ± SD, n=9), respectively, and was not significantly affected by the addition of $CaCl_2$ and/or ATP. HETE produced by 12,000 x g sediment of sonicated platelets was negligible (< 1 % of the product by intact platelets). One of the 7 patients showed no detectable lipoxygenase activity both in intact platelets and in any subcellular fractions, while the other 6 patients showed reduced lipoxygenase activities in all subcellular fractions as well as in intact platelets: HETE produced by intact platelets, F-I, F-II and F-III was 78.8 ± 112.3, 59.1 ± 36.3, 37.0 ± 18.9 and 17.7 ± 15.8 (n=6), respectively. The addition of $CaCl_2$ significantly increased HETE production only by the patient's F-I ($p < 0.02$), while ATP showed no significant effect in any experiments.
Thus, it was shown that lipoxygenase activities were not fully exhibited in intact platelets with the deficient enzyme activities and that F-I could produce more HETE than intact platelets especially in the presence of $CaCl_2$ only in the case of such patient's platelets.

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EFFECT OF ATRIAL NATRIURETIC POLYPEPTIDES ON PLATELET FUNCTION.

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Atrial natriuretic polypeptides (ANP) have been shown to possess a potent diuretic and natriuretic activity, and medicated to patients with heart insufficiency as a drug to be mediated by cGMP accumulation in glomeruli. A existence of receptors for ANP have recently been reported in human platelet. But, whether ANP has a direct effect on platelet function remains to be known.

Single stimulation of ANP in any concentration did not induce aggregation in neither platelet rich plasma, nor washed platelets. Also no effect of pretreatment with ANP was observed against aggregation triggered by known mediators of platelet activation (Thrombin, ADP, Epinephrine, Collagen) using platelet rich plasma and washed platelets.

Therefore, biochemical parameters such as cyclic nucleotides (cAMP, cGMP), phosphatidylinositol hydrolysis and protein phosphorylation, leading to the early stage of platelet activation were examined to investigate the effect of ANP in receptor linked transducing mechanism. Neither cyclic nucleotides accumulation nor [^{32}P] phosphatidic acid production were detected in platelets treated with ANP. ANP caused a small increase of ^{32}P incorporation into M$_r$30K protein, but no change on the level of phosphorylation of 47K, 20K protein (Imaoka, T. and Haslam, R.J., J.Biol.Chem.258,11404, 1983) was observed.

These results clearly suggested that ANP binding with membrane receptor was not linked with adenylate cyclase, ganulate cyclase and phosphatidylinositol phosphate turnover in human platelet, maybe because of too few numbers of ANP receptor. Mechanism of 30K protein phosphorylation and Ca^{++} mobilization are important subjects for future study. (supported by MESC of Japan)

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EFFECTS OF HUMAN ATRIAL NATRIURETIC PEPTIDES ON SECRETION REACTION IN HUMAN PLATELETS.

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Human atrial natriuretic peptide (h-ANP) has vasodilating and natriuretic properties, and inhibits smooth muscle contraction, renal renin secretion and adrenal aldosterone release. Although Schiffrin has reported that human platelets have receptors for ANP, its effects in platelets are not established in vivo. We therefore investigated the influence of h-ANP on secretion reaction in human platelets. Eight healthy subjects, males, aged 20 to 24 years, donated blood for the study. Citrated platelet-rich plasma (PRP) was incubated with or without h-ANP at 37 C for 2.5 minutes. The samples of 0.5 ml PRP then used to measure ADP induced aggregation, ATP release reaction and ^{14}C-serotonin release reaction. H-ANP, at concentration of $1x10^{-6}$M, decreased ADP induced aggregation (after h-ANP: 77.4±9.7 % of control aggregation), and inhibited ATP release reaction (after h-ANP: 31.8 ± 13.1 %). Serotonin release reaction induced by ADP was also inhibited (control: 15.3 ± 2.2%, after h-ANP: 8.3 ± 0.5 %). The inhibitory effect of h-ANP on aggregation and secretion reaction was maximal by 3 minutes. These data suggest that h-ANP inhibits secretion reaction in human platelets.

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ALTERATIONS OF THE ACTIN STATUS OF PLATELETS AFFECTS THE FUNCTIONAL BEHAVIOUR OF THE CELL.

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Many platelet responses to agonists involve contractile proteins. Particularly, the status of the most abundant protein actin is changed in motile and contractile events. We have studied the effects of SH-reagents on platelets and found significant effects of a SH-oxidizing agent on the actin organization which are neutralized following the addition of a disulfide-reducing compound. SH-oxidation of intact platelets was achieved by incubation with diamide (azodicarboxylic acid-bis-dimethylamide). The F-actin of those cells is increased and the filaments became centralized indicating a disturbance of the membrane-cytoskeleton interaction. Treatment of SH-oxidized platelets with 2-mercaptopropionylglycine which reduces disulfides resulted in a return of F-actin levels to those seen in untreated cells. The actin organization of platelets is discussed with regard to the altered functional behaviour of diamide-treated cells.