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POTENTIATION OF ANTI-AGGREGATING PROSTAGLANDINS BY TICLOPIDIN. C. Bonne, B. Martin and F. Regnault
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Ticlopidin (T) is a potent inhibitor of platelet aggregation which enhanced the platelet sensitivity to anti-aggregating prostaglandins such as PGE1. The goal of this study was to examine the site of action of T in the rat platelets after in vivo administration, in respect to the PGE1 mechanism of action.

Male adult Wistar rats were orally administered at the dose of 200 mg/kg, 20 and 4 hours before blood collection. The binding of (3H)-PGE1 to platelets from controls and treated rats was determined by filtration technique. The c AMP formation was measured by protein binding assay in aspirin-washed platelet suspension or in lysate after stimulation by PGE1, adenosine or NaF.

Since potentiation of PGE1 by T occured in aspirin-washed platelets, it is independent of the ability of the cells to synthesise prostaglandins. On the other hand, PGE1-receptor binding is not affected by T treatment and potentiation of PGE1 effect is not specific. Moreover, basal adenylate cyclase activity is increased in platelet particulate fraction of treated rats,but NaF stimulation is not enhanced.

These results suggest that T activates the adenylate cyclase systems by an action on the coupler subunit of the enzyme just as guanosine triphosphate does.

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THE IN VITRO EFFECT OF TICLOPIDINE ON FIBRINOGEN AND FACTOR VIII BINDING TO HUMAN PLATELETS. H. Lee, R.C. Paton and C. Ruan. INSERM Unité 150, Director J.P. Caen, Hôpital Lariboisière & Service de Nutrition et Endocrinologie, Hôpital St-Louis, Paris, France.

Ticlopidine is an anti-aggregating drug whose mode of action is not yet fully understood. Its multiple effects on platelet function include prolonged bleeding time, reduction in primary and secondary waves of ADP-induced aggregation and inhibition of collagen and thrombin-induced aggregation. We have studied the in vitro effects of ticlopidine on fibrinogen binding induced by ADP and adrenaline as well as Factor VIII/vWF binding induced by ristocetin.

^{125}I fibrinogen binding was measured in suspensions of freshly-washed normal platelets stimulated by 10 µM ADP or 10 µM adrenaline. The binding of ^{125}I-Factor VIII/vWF in the presence of 1 mg/ml ristocetin was measured in both washed and paraformaldehyde-fixed platelets. Ticlopidine at final concentrations of 200, 100, 50 and 25 µM inhibited both ADP and adrenaline-induced fibrinogen binding in a dose-dependent manner. The mean % inhibition of ADP-induced fibrinogen binding was 82, 73, 42 and 32 respectively. The mean % inhibition of adrenaline-induced fibrinogen binding was 86, 82, 60 and 35 respectively. In contrast, the Factor VIII/vWF binding was unaffected by ticlopidine at all concentrations except at 200 µM using fresh platelets where a slight inhibition (19 %) was observed.

These results suggest that ticlopidine either inhibits platelet activation and consequently fibrinogen binding, or inhibits the binding directly, presumably by having an effect on the specific configuration of the platelet membrane required for normal fibrinogen binding.

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ANTIPLATELET EFFECT OF A NEW COMPOUND, IMIDAZO[1,2-a]-THIENOPYRIMIDIN-2-ONE : A CYCLIC AMP MEDIATED PHENOMENON. S. Ashida, K. Sakuma and Y. Abiko. Laboratory of Biochemistry, Research Institute, Daiichi Seiyaku Co., Ltd. Edogawa-ku, Tokyo 132, Japan

The effect of a new compound, 1,2,3,5,6,7,8,9-Octahydro-[1]benzothieno[2,3-d]imidazo[1,2-a]pyrimidin-2-one hydrochloride (DH-6471), on cAMP metabolism and aggregation of platelets was studied. In vitro, DH-6471 inhibited platelet aggregation (both the 1st and 2nd phases) induced by ADP, collagen, thrombin, arachidonic acid and PGG_2-TXA_2 mixture in PRP from various animal species including human at concentrations (IC_{50}) ranging from 0.07 to 8 µM. It inhibited ADP- and collagen-induced platelet aggregation ex vivo in rats following oral doses as low as 0.3- 1 mg/kg.

The compound was found to be a highly selective inhibitor of platelet low Km cAMP phosphodiesterase (Ki=0.025 µM), when tested with enzyme fractions separated by DEAE-cellulose chromatography. It did not significantly affect basal or PGE_1(0.1-1 µM)-stimulated cAMP level of platelets at a concentration of 1 µM where platelet aggregation and the low Km PDE were markedly inhibited. However, both basal and PGE_1-stimulated accumulations of cAMP in the platelet membrane fraction were increased by DH-6471 at 1 µM when the isolated membrane fraction was incubated with ATP-Mg^{2+}.

Studies with several PDE inhibitors including papaverine, dipyridamole and DH-6471-related compounds showed a close correlation between their ability to inhibit the low Km PDE or to increase cAMP accumulation in the membrane fraction and their inhibitory effect on platelet aggregation. On the other hand, their potency to inhibit high Km cAMP-PDE(cGMP-PDE) and to increase cAMP level in whole platelets was poorly correlated to their inhibitory activity in platelet aggregation.

These results suggest that some small but local changes in platelet cAMP may be involved in the regulation of platelet aggregation, particularly primary aggregation.

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PIRACETAM: A NEW PLATELET SUPPRESSING DRUG. R.L. Bick, J. Fareed, and V. Skondia. San Joaquin Hematology Oncology Medical Group, Bakersfield, California, UCLA Center for the Health Sciences, Los Angeles, California, Loyola University School of Medicine, Chicago, Illinois, and UCB Pharmaceuticals, Brussels, Belgium.

Piracetam, 2-Oxy-1-pyrrolidine Acetamide is a cyclic derivative of gamma-aminobutyric acid and has recently undergone successful human trials as a platelet-suppressing agent. These investigations were designed to study the mechanism of action of Piracetam in suppressing platelet function in human subjects. Twenty-eight subjects were studied; these individuals ingested Piracetam at 9.6 gm/day in four divided doses. Template bleeding times, platelet adhesion (Bowie technique) and platelet aggregation to collagen, ristocetin, serotonin, ADP, epinephrine, and arachidonic acid was performed before and one week after beginning ingestion of this drug. All individuals demonstrated prolongation of the template bleeding time; the average prolongation was 6 min. over baseline bleeding times. Platelet adhesion became abnormal in the majority of individuals during ingestion of Piracetam, but the degree of abnormality was not consistent. However, consistent aggregation abnormalities were induced by Piracetam. All individuals showed absence of the second wave of epinephrine at 2.7 uM and 5.4 uM, all individuals demonstrated abnormal aggregation to low concentration ADP (2.0 uM) and most were abnormal to high concentration ADP (6.6 uM). In addition, all individuals demonstrated absence of arachidonic acid-induced aggregation after ingestion of Piracetam. No consistent abnormalities of collagen or ristocetin-induced aggregation were noted. The results of this study suggest that Piracetam has at least two sites of action in suppressing platelet function. One site of action appears to be inhibition of thromboxane systhesis at some, as yet undetermined, site in the platelet prostaglandin synthetic pathway, and the other mechanism appears to be inhibition of release by platelet dense bodies. It is suggested that the presence of at least two sites of action may make this non-toxic agent a highly useful antiplatelet drug.