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THE INFLUENCE OF SOME HYPOTENSIVE DRUGS ON THE SEROTONERGIC MECHANISMS IN RATS PLATELETS.

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Serotonin (5HT) is a vasoactive amine that has been reported to be involved in a number of forms of circulatory failure. The 5HT content and metabolism in platelets is changed in hypertension and in peripheral arteriolar diseases. The present study concerns the effect of verapamil (VER), propranolol (PRO) and captopril (CAP) - drugs having different hypotensive mechanisms of action, on serotonergic mechanisms in rat blood platelets. In vitro, VER produced noncompetitive inhibition of ^{14}C-5HT uptake (IC_{50}=8.2μM), PRO inhibited the uptake in a competitive fashion (K_m=0.9μM), whereas CAP was ineffective. Only PRO released (24%) radioactive 5HT from incubated platelets. Inhibition of amine uptake was also obtained when platelets were prepared from rats pretreated with VER (10 mg.kg^{-1}), PRO (5 mg.kg^{-1}) or CAP (10 mg.kg^{-1}). Moreover, the concentration of endogenous 5HT in blood platelets was reduced after VER and PRO administration. Platelets aggregation induced by ADP was inhibited by VER and CAP. They also diminished the potentiating effect of 5HT on ADP-induced platelet aggregation. It can be concluded that these effects may be a secondary mechanism of action in vivo. Thus "serotonergic component" of studied drugs should be taken under consideration at least in therapy of hypertension.

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EFFECTS OF FEVERFEW EXTRACT AND PARTHENOLIDE ON PLATELET SECRETION.

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Feverfew (Tanacetum parthenium) is used for prophylaxis of migraine and it had been suggested that the plant may have antithrombotic potential. We have prepared extracts from the leaves of feverfew and have demonstrated inhibition of ^{14}C-5HT secretion in platelet-rich plasma induced by the phorbol ester PMA, 1-oleoyl-2-acetyl-sn-glycerol (OAG), arachidonic acid, the thromboxane analogue U46619, adrenaline, collagen and ADP. The effects of a solution of parthenolide (an α-methylenebutyrolactone isolated from feverfew) were determined in parallel. Both feverfew extract (FE) and parthenolide inhibited ^{14}C-5HT release in a concentration-dependent manner and the effectiveness depended on the nature of the aggregating agent used. Both FE and parthenolide were most effective as inhibitors of the secretion induced by PMA and OAG. When we compared the concentrations of FE and parthenolide which gave 50% inhibition of secretion for all the agents tested, a good correlation was found (r= 0.936). Further studies showed that feverfew extract and parthenolide inhibit release of β-thromboglobulin from platelets as well as ^{14}C-5HT. FE did not cause liberation of LDH. Inhibition of secretion by FE appears to be irreversible since washing platelets after treatment did not restore secretory activity.

The structure of parthenolide suggests that it can alkylate sulphydryl (SH) groups. When agents containing SH groups (e.g. cysteine) were added to FE, anti-secretory activity was reduced. We also obtained a considerable decrease in the number of acid-soluble SH groups in platelets treated with feverfew extract or parthenolide at concentrations which inhibit secretion. However there was a less marked decrease in the number of acid-insoluble SH groups. FE itself does not induce formation of disulphide-linked proteins but such proteins were formed when platelets were activated in the presence of FE, probably as a result of decreased glutathione levels.

We conclude that parthenolide or parthenolide-like compounds are responsible for the anti-secretory effects of FE, and that alkylation of sulphydryl groups in platelets may be involved.

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HUMAN PLATELET ACTIVATION BY BACTERIAL PHOSPHOLIPASE C: MECHANISM OF INHIBITION BY FLURAZEPAM.

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We have shown earlier that flurazepam inhibits human platelet aggregation and serotonin secretion induced by bacterial phospholipase C (BPLC, Thromb. Res. 38, 361-374, 1985). This study was conducted to examine the mechanism(s) of inhibitory action of flurazepam. Only 15 uM and 11 uM flurazepam were required to inhibit platelet aggregation and serotonin secretion by 50%. In a platelet free system, BPLC hydrolyzed ^{14}C-phosphatidylcholine (^{14}C-PC) in a time- and concentration-dependent manner in the presence of calcium ions. Flurazepam had no effect on BPLC-induced hydrolysis of ^{14}C-PC. Incubation of ^{14}C-arachidonic acid labelled platelets with BPLC produced diacylglycerol(DAG) in a time- and concentration-dependent manner. Flurazepam did not inhibit DAG production by BPLC. However, prostaglandin E_1 and paranitrophenolphosphorylcholine inhibited DAG production by 20% and 75% respectively. Platelet cytosolic fraction, containing phosphatidylinositol-specific PLC (PI-PLC), hydrolyzed ^{3}H-phosphatidylinositol (^{3}H-PI) in a concentration-dependent manner. Flurazepam did not inhibit hydrolysis of ^{3}H-PI by PI-PLC. BPLC caused phosphorylation of 47,000 Dalton protein (P47) in ^{32}P-labelled platelets. Flurazepam did not inhibit phosphorylation of P47 in the first five minutes of incubation. However, flurazapam completely blocked phosphorylation of P47 by seven minutes. In other experiments, flurazepam inhibited platelet aggregation induced by ionomycion, a calcium ionophore, in a concentration-dependent manner. These data lead us to suggest that flurazapam does not inhibit BPLC-induced platelet activation by inhibiting the action of BPLC or PI-PLC on platelet phospholipids or DAG production. However, the ability of flurazepam to inhibit ionomycin-induced platelet aggregation indicates that it may be blocking BPLC-induced platelet aggreagtion by interfering with the influx of calcium ions into platelets. (Supported in part by the American Osteopathic Association, The Baker Award from Ohio University and the OUCOM).

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INFLUENCE OF LOMUSTIN ON SOME PLATELET FUNCTIONS IN VITRO.

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Lomustin (CCNU) is a cytostatic drug that is used in the treatement of some forms of solid tumors and hemoblastosis. The most important form of toxicity associated with its use consists of thrombocytopenia often associated with hemorrhagic syndrome. Lomustin may exert its action on the formation of platelets, but it may also interfere with platelets themselves.

In the present study its influence on the platelet function was investigated using a wide battery of tests.

Platelet-rich plasma was incubated with lomustin (1,5 to 15 μg/ml and 30 minutes at 37°C) in vitro.

This inhibited strongly aggregation by ADP ($2x10^{-6}$M), adrenaline ($2x10^{-6}$M) and collagen (15 μg/ml). Platelet factor 4 release was almost completly inhibited independently of the inducer used. ^{14}C-serotonin release was decreased by approximately 70% when incubation proceeded in the presence of 1,5 μg/ml of lomustin, and completly at higher concentration. Platelet factor 3 availability was also significantly impaired. Reptilase clot retraction was diminished, regardless of inducer used. In the presence of N-ethyl maleimide (1 mmol) significantly lower malondialdehyde was produced in platelets incubated with lomustin, than in that from normal controls. After addition of arachidonic acid (1 mmol), the platelet synthesized less thromboxane B2. Lomustin did not interfere with coagulation factors and did not induce overt fibrinolysis.

Its is concluded that lomustin acts as an inhibitor of platelet functions and can induce an acquired thrombocytopathy.

Thus impairement of platelet functions might play a part in hemorrhagic complications accompanying in some cases lomustin therapy.

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