

13-AZAPROSTANOIC ACID POTENTIATES PROSTACYCLIN-INDUCED PLATELET DEAGGREGATION. L. V. Parise, D. Venton and G. C. Le Breton. Depts. of Pharmacology and Med. Chemistry, Univ. of Ill. at the Med. Ctr., Chicago, USA

13-Azaprostanoic acid (13-APA), a specific thromboxane/endoperoxide receptor antagonist, reverses platelet aggregation stimulated by the endoperoxide analog U46619. The present report demonstrates that 13-APA also potentiates prostacyclin (PGI₂) reversal of U46619-induced aggregation. Human platelet-rich plasma was aggregated with 3×10^{-6} M U46619. Deaggregation was induced 2 min. subsequent to the addition of aggregating agent and was measured over a 3 min. period. Concentrations of 13-APA (4×10^{-4} M) and PGI₂ (4×10^{-9} M) were chosen such that each agent individually induced approximately 20% deaggregation. Addition of half of the above concentrations of these agents i.e. 2×10^{-4} M 13-APA plus 2×10^{-9} M PGI₂ resulted in 62% deaggregation, demonstrating that the observed response was supraadditive. Only 8% deaggregation was induced by 2×10^{-4} M 13-APA alone and 0% by 2×10^{-9} M PGI₂ alone. PGI₂ causes platelet deaggregation presumably through elevation of cAMP. 13-APA, however, did not increase cAMP levels even at concentrations of 13-APA as high as 1.2×10^{-3} M i.e. 9.8 ± 1.3 pmoles/ml for control and 10.8 ± 1.2 for 13-APA. Nevertheless it is possible that the observed potentiation of deaggregation was the result of 13-APA facilitating PGI₂ stimulation of adenylate cyclase. Measurement of cAMP during deaggregation, however, showed no significant difference between treatment with PGI₂ alone and treatment with PGI₂ plus 13-APA i.e. 11.3 ± 0.4 pmoles/ml for control, 11.4 ± 0.3 pmoles/ml for 13-APA, 16.1 ± 0.5 pmoles/ml for PGI₂ and 16.5 ± 0.8 pmoles/ml for PGI₂ plus 13-APA. These results clearly establish that 13-APA and PGI₂ deaggregate platelets by distinctly separate mechanisms. In this regard we propose that PGI₂ causes platelet deaggregation by stimulating intraplatelet calcium sequestration through a cAMP dependent process. 13-APA, on the other hand, blocks the ability of U46619 to mobilize intraplatelet calcium. The combination of these two mechanisms presumably results in the observed potentiation of deaggregation.

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ARACHIDONIC ACID-INDUCED PLATELET AGGREGATION INDEPENDENT OF CYCLOOXYGENASE AND LIPOXYGENASE. J. MacLouf, S. Levy-Toledano, H. de la Baume, R.M. Hardisty and J.P. Caen
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It is generally admitted that arachidonic acid (20:4 W6) can stimulate platelet aggregation after transformation into prostaglandin G₂ and H₂ further converted in thromboxane A₂. This action is prevented by cyclooxygenase inhibitors. Washed platelets were isolated on metrizamide gradient and resuspended in a Ca²⁺ free buffer. Their stimulation by C20:4W6 was followed by (¹⁴C)-serotonin (5HT) release, thromboxane (TX) synthesis and a change of light transmission, not dependent on aggregation, accompanied by a slight lysis (14%). The addition of external Ca²⁺ suppressed lysis and allowed the formation of aggregates. Under these conditions, cyclooxygenase inhibitors like acetyl salicylic acid, indomethacin or flurbiprofen totally suppressed TX synthesis without preventing platelet aggregation or (¹⁴C)-5HT release. Other C 20 polyunsaturated fatty acids could not substitute for C20 : 4W6-induced aggregation and Ca²⁺ was found to be the most effective divalent cation for protection of the cell against lysis as well as in allowing the platelet to aggregate in the absence of TX formation. The use of the lipoygenase inhibitor BW 755C did not prevent C20 : 4W6 - induced aggregation of aspirin-treated platelets, suggesting that the phenomenon was independent of this pathway also. The total suppression of oxidative metabolism with these inhibitors was verified by the analysis of eicosanoids using glass capillary column-gas chromatography. It is suggested that under these conditions, C20 : 4 W6-induced platelet aggregation might be due to an ionophoric action of C20 : 4W6 in the absence of oxidation.

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13-AZAPROSTANOIC ACID OR INDOMETHACIN INHIBIT TXA₂/PGH₂ STIMULATED RELEASE OF CALCIUM FROM ISOLATED PLATELET VESICLES. J. P. Rybicki, D. L. Venton, G. C. Le Breton. Departments of Pharmacology and Medicinal Chemistry, University of Illinois Medical Center, Chicago, IL USA.

13-azaprostanoic acid (13-APA) acts as a direct antagonist of the human blood platelet thromboxane/endoperoxide receptor. Previous studies have suggested that arachidonic acid (AA) metabolites e.g. thromboxane A₂ (TXA₂) and prostaglandin H₂ (PGH₂) can mobilize Ca²⁺ from Ca²⁺ accumulating vesicles derived from the platelet dense tubular system. In the present study we investigated the effect of 13-APA on this Ca²⁺ mobilization process. Platelet vesicles were prepared as previously described with the exception that oxalate was not incorporated into the vesicles as a precipitating anion. ⁴⁵Ca uptake was determined by millipore filtration and isotope counting of the filter paper. ³H-sucrose was used as an index of extravesicular Ca²⁺. TXA₂ production by the vesicles was measured by radioimmunoassay of TXB₂. Vesicles were incubated with 5×10^{-5} M Ca²⁺ before addition of AA. Ca²⁺ uptake and TXB₂ production were determined before and at 3, 9, and 16 min following the addition of AA. It was found that the vesicles accumulated 10 nmoles of Ca²⁺ per mg of protein under steady-state conditions. Addition of AA (2.5×10^{-5} M) resulted in the release of $19 \pm 6.2\%$, $34 \pm 4.8\%$ and $42 \pm 5.1\%$ of accumulated Ca²⁺ at 3, 9 and 16 min respectively. Pretreatment of the vesicles with indomethacin (4×10^{-6} M) completely inhibited AA-induced release and reduced TXB₂ production by 82%. Pretreatment of the vesicles with 13-APA (2×10^{-5} M) also completely inhibited Ca²⁺ release. In this case, however, there was no inhibition of TXA₂ synthesis, i.e. 56 ± 9 ng TXB₂ per mg protein in the control and 64 ± 12 ng TXB₂ per mg protein with 13-APA. These results confirm previous studies that AA must be metabolized to TXA₂/PGH₂ in order to release Ca²⁺ from isolated membrane vesicles. The finding that 13-APA blocks TXA₂/PGH₂ stimulated release of Ca²⁺ suggests that this release process is mediated through a specific receptor interaction. (Supported by a grant in aid AHA 79895.)

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LACK OF INHIBITION OF THROMBOXANE PRODUCTION DESPITE INHIBITION OF PLATELET FUNCTION BY 1,3-BIS(2-CHLOROETHYL)-1-NITROSOUREA (BCNU). R. McKenna, T. Ahmad, A. Prancan, D. Simon and H. Frischer. Departments of Medicine and Pharmacology, Rush University, Chicago, IL. 60612, U.S.A.

We have previously shown that BCNU inhibits human platelet glutathione reductase (GSSG-R) prior to inhibiting platelet function; since thromboxane production is important in platelet function, we evaluated the effect of BCNU induced inhibition of GSSG-R on platelet thromboxane production.

Control platelet GSSG-R activity was 0.091 μ moles NAD(P)H oxidized min⁻¹mg⁻¹ protein at 37°C (± 0.015 S.D.; n=9); inhibition was detectable at 10^{-7} M BCNU (70% of control) with a >90% inhibition at and above 10^{-5} M BCNU. Platelet aggregation in response to 1.5×10^{-3} M Arachidonic acid (AA), 10μ M epinephrine, 6μ g/ml equine collagen and 3μ M ADP were inhibited at 10^{-3} M BCNU and abolished at 10^{-4} M BCNU.

BCNU (10^{-3} M) did not affect the increase in oxygen consumption induced by AA. Using the rabbit aorta superfusion bioassay for thromboxane A₂ (TXA₂), threshold concentrations of AA in 10^{-5} M and 10^{-4} M BCNU platelets resulted in an increased measure of aortic tension 13.5 ± 9.4 mm S.D. (n=6) and 23.2 ± 9.5 mm respectively, compared with control values of 4.5 ± 2.4 . Acetylsalicylic acid (5×10^{-4} M) inhibited the contraction: 1.7 ± 1.1 (n=5). The conversion of ¹⁴C AA to thromboxane B₂ (TXB₂) and PGE₂, as measured by radio TLC, was not decreased in BCNU treated platelets. There is a significant increase in TXB₂ ($p < 0.05$; n=4) and in the ratio of TXB₂:PGE₂ in platelets treated with 10^{-4} M BCNU and 10^{-3} M imidazole when compared to platelets treated with imidazole alone.

In conclusion BCNU induced inhibition of platelet GSSG-R and platelet function occurs despite preservation of thromboxane production.