
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 AA (20:4)-induced aggregation. Human platelet-rich plasma was aggregated with 3 x 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 (6 x 10^{-6} M) and PGI2 (6 x 10^{-7} 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 x 10^{-6} M 13-APA plus 2 x 10^{-6} M PGI2 resulted in 62% deaggregation, demonstrating that the observed response was supra-additive. Only 8% deaggregation was induced by 2 x 10^{-6} M 13-APA alone and 6% by 2 x 10^{-6} 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 x 10^{-3} M (i.e. 9.8 ± 1.3 pmol/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 pmol/ml for control, 11.1 ± 0.3 pmol/ml for 13-APA, 16.1 ± 0.5 pmol/ml for PGI2 and 16.5 ± 0.8 pmol/ml for PGI2 plus 13-APA. These results clearly establish that 13-APA and PGI2 deagregate 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.


It is generally admitted that arachidonic acid (20:4 W6) can stimulate platelet aggregation after transformation into prostaglandin (PG) and thromboxane (TX) further converted in thromboxane (TX) and prostaglandin (PG). Stimulation of PGH2 (the product of cyclo-oxygenase and lipooxygenase) was measured over a 3 min. period. Other prostaglandin (PG) or thromboxane (TX) synthesis was blocked by aspirin or ibuprofen, respectively. Under these conditions, cyclo-oxygenase inhibitors like acetyl salicylic acid, indomethacin or flurbiprofen totally inhibited TXA2 at 3, 9 and 16 min respectively. Pretreatment of the vesicles with indomethacin (6 x 10^{-6} M) completely inhibited AA-induced release and reduced TXB2 production by 82%. Pretreatment of the vesicles with 13-APA (2 x 10^{-7} M) also completely inhibited TXA2 release. In this case, however, there was no inhibition of TXA2 synthesis, i.e. 55 ± 9 ng TXB2 per mg protein in the control and 12 ± 11 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 78-895.)

LACK OF INHIBITION OF THROMBOXANE PRODUCTION DESPITE INHIBITION OF PLATELET FUNCTION BY 1,3-BIS(2-CHLOROETHOXY)-L-1-NITROGUATA (BCNU). R. Neffena, T. Ahmad, A. Francan, D. Simon and B. Frichee. Deps. 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 inhibition of GSSG-R on platelet thromboxane production.

Control platelet GSSG-R activity was 0.191 nmoles NADPH oxidized min^{-1}mg^{-1} protein at 37°C (± 0.015 S.D.; n=9). Inhibition was detectable at 10^{-7} BCNU (70% of control) with a >90% inhibition at and above 10^{-6} BCNU. Platelet aggregation in response to 1.5 x 10^{-6} Archidonic acid (AA), 10 μM epinephrine, 6 μg/ml equine collagen and 3 μM ADP were inhibited at 10^{-6} BCNU and abolished at 10^{-5} BCNU.

BCNU (10^{-5}) did not affect the increase in oxygen consumption induced by AA. Using the rabbit aorta superfusion bioassay for thromboxane (TXA2), threshold concentrations of AA in 10^{-5} and 10^{-4} BCNU platelets resulted in an increased measure of aortic tension (p<0.05; n=6) and platelet function occurs despite preservation of thromboxane production.