Vol. 100, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1981 Pages 184-190

INHIBITION OF HUMAN PLATELET THROMBOXANE SYNTHETASE BY 11a-CARBATHROMBOXANE A_2 ANALOGS

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Received March 24,1981

SUMMARY

Three 11a-carbathromboxane A_2 analogs were synthesized and evaluated for "thromboxane A_2-like" biological activity in human platelets and rat aortic strips. All three analogs were potent inhibitors of either prostaglandin H_2 or arachidonic acid-induced human platelet aggregation, and proved to be both receptor level antagonists of thromboxane A_2 and thromboxane synthetase inhibitors.

The three 11a-carba analogs were devoid of either agonist or antagonist activity on rat aortic strips, suggesting that the thromboxane A_2 receptor in platelets exhibits different binding characteristics than the thromboxane receptor in vascular smooth muscle.

INTRODUCTION

The isolation and purification of the prostaglandin endoperoxides by Hamberg et al. (1) and Nugteren and Hazelhof (2), and the subsequent publication of the structure and biological activity of thromboxane A_2 (TXA_2) (3) has stimulated the search for stable "thromboxane-like" molecules.

The first stable molecules with "thromboxane-like" biological activities (stimulation of human platelet aggregation and constriction of rabbit aorta) were more structurally related to endoperoxides than TXA_2 (4). Subsequent modifications of these original analogs have produced new agonists as well as thromboxane synthetase inhibitors and antagonists (5-7).

Recently a number of carbathromboxane analogs have been synthesized where carbon has been substituted for one or more of the ring oxygens of TXA_2 (8-11).

In this paper we report the biological activity of three carbathromboxane molecules which are both receptor level antagonists of TXA_2 and

0006-291X/81/090184-07$01.00/0

thromboxane synthetase inhibitors. Although they block either arachidonic or PGH_2-induced human platelet aggregation, they are devoid of agonist or antagonist activity on blood vessels.

METHODS AND MATERIALS

Arachidonic acid was purchased from Nu-Chek Prep. (Elysian, MN). [^{3}H] TXB_2 (125 Ci/mMole) was purchased from New England Nuclear (Waltham, MA). The carbathromboxane analogs were synthesized by minor variants of previously published methods (10).

Human platelet rich plasma (PRP) was prepared from fresh whole blood collected in 3.8% trisodium citrate (1:9, v/v), followed by centrifugation at 200 x g for 10 min at room temperature. Platelet aggregation induced by 0.3 mM arachidonic acid was monitored at 37° with a Payton Aggregometer with constant stirring at 1100 rev/min. Platelets were incubated for 2 min at 37° prior to the addition of arachidonic acid, and the various carbathromboxane analogs were added to the platelets during this 2 min period. Thromboxane B_2 formed during the aggregation response was measured according to Fitzpatrick et al. (12), and reported as ng/ml PRP.

Aorta contracting activity of the various carbathromboxane analogs was measured using spirally cut strips of aorta according to the method of Furchgott (13). The strips were placed in tissue baths containing 10 ml of Kreb's bicarbonate buffer at 37.5°C. One end of the tissue was fixed, and the other was attached to an isometric transducer (Grass FT.03) with an initial tension of 2 grams. During a one-hour equilibration period the tissue relaxed to an average basal tension of 1.55 ± .05 grams. Data are reported as the concentration (ng/ml) required to give half-maximal contraction (ED_{50}).

RESULTS AND DISCUSSION

Three carbathromboxane A_2 analogs were synthesized and evaluated for intrinsic "TXA_2-like" biological activity. The structures of the three analogs as well as authentic TXA_2 are shown in Figure 1. 11a-Carba TXA_2

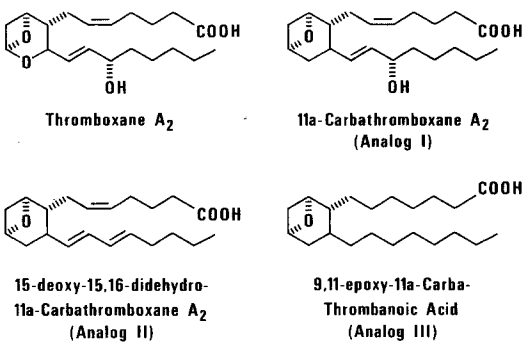

Figure 1. Structure of authentic thromboxane A_2 and 11a-carbathromboxane A_2 analogs.

(analog I) is identical to authentic TXA_2 except a carbon atom has been substituted for the 11a oxygen. Analog II is a 15-deoxy variation of analog I, with an additional double bond at the 15,16 position, and analog III is a fully saturated 15-deoxy molecule.

Due to the similarity in structure between authentic TXA_2 and these analogs, it was surprising to find that all three molecules suppressed either PGH_2 or arachidonic-induced human platelet aggregation. A typical profile of activity for the carba analogs (using 11a-carbathromboxane A_2 as an example) is shown in Figure 2. In addition to arachidonic acid and PGH_2, 11a-carba TXA_2 also inhibited aggregation induced by the stable endoperoxide analog 11,9-epoxymethanoprosta-5,13 dienoic acid (Figure 2). Previous work has shown that 11,9-epoxymethano-induced aggregation was not blocked by specific thromboxane synthetase inhibitors, but was blocked by receptor level antagonists of TXA_2 (7). Therefore, the carba analogs were either selective receptor level antagonists of TXA_2, or they were both receptor level antagonists and TXA_2 synthetase inhibitors.

To assess directly whether or not the analogs were inhibitors of the TXA_2 synthetase we measured the stable hydrolysis product of TXA_2, thromboxane B_2 (TXB_2) levels during arachidonic acid-induced human platelet aggregation. All of the analogs dose dependently inhibited TXB_2 formation (Figure 3). While TXB_2 levels were falling, PGE_2 levels actually increased

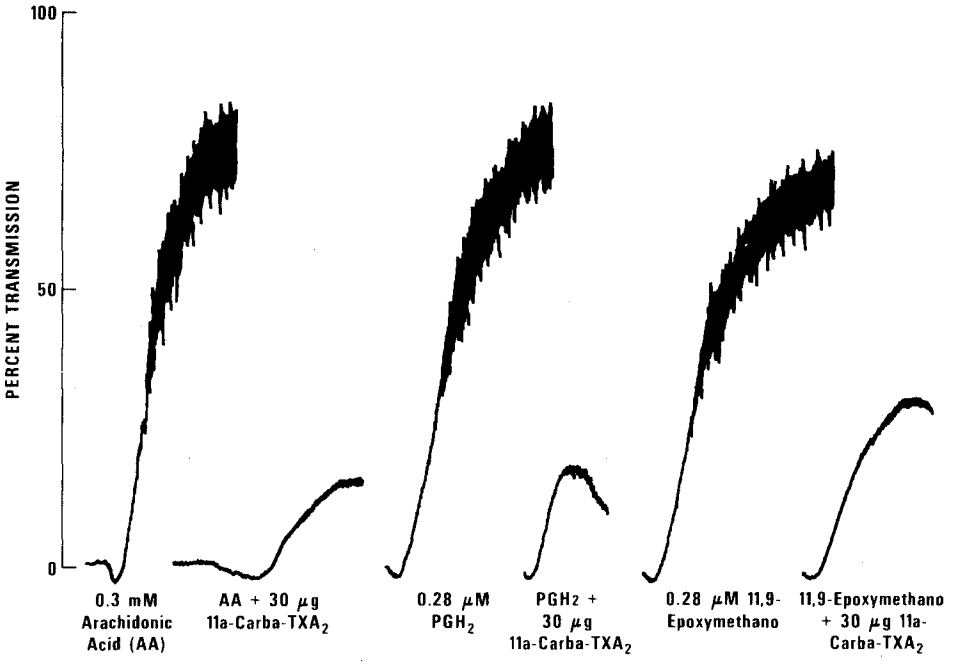

Figure 2. Inhibition of human platelet aggregation by 11a-carbathromboxane A_2.

Human PRP was preincubated for 2 min with 30 μg/ml of 11a-carbathromboxane A_2 and then challenged with the appropriate agonist at the indicated concentration.

(data not shown). The inhibition of TXB_2 synthesis correlated with the inhibition of aggregation. Analog II was the most potent inhibitor followed by 11a-carba TXA_2 (analog I), and the fully saturated thrombanoic acid molecule (analog III) was the least active (Figure 3). Parenthetically, none of the carba analogs influenced prostacyclin biosynthesis in cultured human umbilical vein endothelial cells (data not shown).

Since the thromboxane analogs proved to be inhibitors of platelet aggregation, they were tested for potential agonist activity on rat aorta. Authentic TXA_2 constricts aorta, but none of the analogs demonstrated any agonist activity on isolated rat aortic strips (Table 1). The analogs were at least 600 times less potent than authentic TXA_2, and none of the analogs effectively antagonized contractions induced by authentic TXA_2 (Table 1).

There have now been several published attempts to synthesize stable analogs with the same structure and biological profile of TXA_2, but none

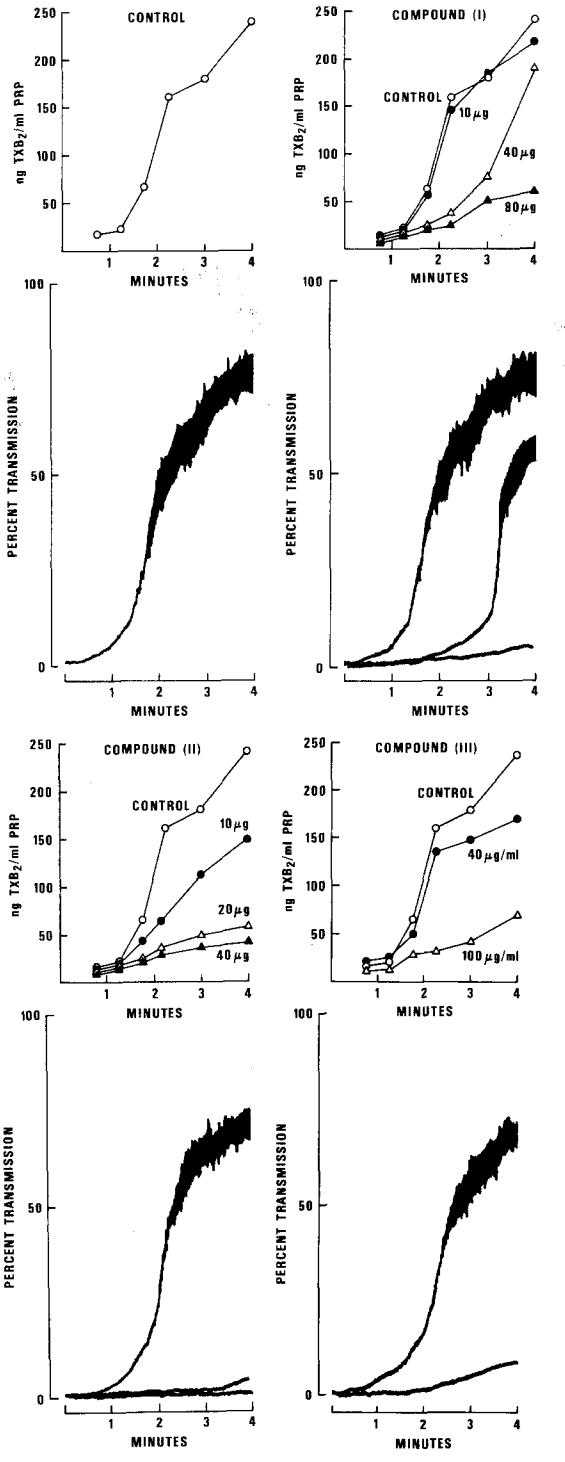

Figure 3. Inhibition of human platelet thromboxane synthesis by 11a-carba-thromboxane A_2 analogs.

Human PRP was preincubated for 2 min with and without the 11a-carba-thromboxane A_2 analogs at the indicated concentrations and then challenged with 0.3 mM arachidonic acid. Samples of PRP were removed and analyzed for TXB_2 levels throughout the aggregation response. The control TXB_2 synthesis is superimposed on all of the traces for reference.

TABLE 1

Rat Aorta Contractile Potency

Molecule	ED_{50} (ng/ml)
Thromboxane A_2	2-5[1]
Prostaglandin H_2	200-400
11a-carbathromboxane A_2 (I)	>3000
15-deoxy-15,16-didehydro-11a-carbathromboxane A_2 (II)	>3000
9,11-epoxy-11a-carbathrombanoate (III)	>3000
Thromboxane A_2 + 3000 ng 11a-carbathromboxane A_2	2-5[2]

[1]Because of the lability of TXA_2 in aqueous solution ($t^{\frac{1}{2}}$ = 32 sec) this value is somewhat variable, and the concentration was determined indirectly by measuring TXB_2 by radioimmunoassay. TXA_2 was generated by incubating human platelet microsomes with PGH_2 (14).

[2]The muscle strips were preincubated for 5 min with 3000 ng/ml of each of the carba analogs and then challenged with authentic TXA_2. Although only the data for 11a-carba TXA_2 is shown, none of the compounds inhibited TXA_2-induced contractions.

have clearly mimicked TXA_2. Although the biological profile of the 9α,11α-methano-TXA_2 has not been published, a preliminary report said it had "biological activity which could not have been predicted" (11). The all-carbon analog synthesized by Nicolaou and associates was reported to be both a receptor level antagonist of TXA_2 and a thromboxane synthetase inhibitor (9). This is analogous to what we found with the 11a-carba analogs. However, unlike our 11a-carba analogs which were devoid of agonist activity on blood vessels, the all-carbon analog was a very potent constrictor of coronary arteries (9). Finally, the pinane analog of thromboxane A_2 was reported to be an antagonist of thromboxane A_2 in both platelets and blood vessels (8).

Why these structurally similar analogs exhibit such diverse biological activities is not clear, but these data highlight the apparent difference(s) between the TXA_2 receptor in human platelets and the TXA_2 receptor in blood vessels. Our initial work with the TXA_2 receptor level antagonist 9,11 epoxyiminoprosta-5,13 dienoic acid showed that compounds could be antago-

nists in platelets, but agonists on blood vessels (7) and more recently Lefer et al. (9) have also reported analogous compounds that block in platelets, but are agonists on blood vessels. More importantly, the pinane-TXA_2 analog reported by Nicolaou et al. (8) and our 11a-carba series suggest that analogs can be synthesized that have a spectrum of biological activities that could be useful in antithrombotic therapy.

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