Stimulating effect of thromboxane B_2 on isolated rat atria[1]

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Summary. Thromboxane B_2 enhanced the beating frequency and the contractile tension of isolated rat atria. Both effects were inhibited by propranolol, by pretreatment with 6-hydroxydopamine, or after inhibition of prostaglandins synthesis with indomethacin and acetylsalicylic acid.

Thromboxane A_2 (TXA_2), a primary endoperoxide metabolite, has been demonstrated to produce a potent platelet proaggregatory effect[2] and to induce constriction of vascular smooth muscle including coronary arteries[3-5]. TXA_2 is a very unstable substance and is rapidly transformed into the more stable thromboxane B_2 (TXB_2). Although it is generally accepted that TXB_2 is devoid of biological activity[6], various studies have documented several actions of TXB_2 on different biological systems[7-11]. Recently Anhut et al.[12] reported that guinea-pig hearts perfused with blood-free medium released substantial amounts of TXB_2. Since vascular tissue has not been shown to synthesize thromboxane the above findings suggest that the myocardium may be the source. Therefore we decided to examine possible influences of TXB_2 on the function of isolated rat atria. In addition, the effects of propranolol, 6-hydroxydopamine (6-OHDA), indomethacin and acetylsalicylic acid (ASA) on atrial responses to TXB_2 were also studied.

Methods. Male albino rats of the Wistar strain were decapitated, and their auricles removed and suspended in a modified Krebs-Ringer Bicarbonate solution gassed with 5% CO_2 in oxygen, maintained at pH 7.4 and 30 °C, with an ionic composition as reported elsewhere[13]. After 1 h of equilibration, initial values of the isometric tension of the spontaneously beating atria (IDT) and of the beating frequency were measured as previously described[14]. Left auricles, driven with slightly suprathreshold (+10%) square pulses of 0.5 msec duration and 3.3 Hz delivered by a conventional stimulator and conveyed to the tissue via 2 platinum electrodes, were also used[13,15]. Cumulative dose-response curves for thromboxane B_2 were constructed for untreated atrial controls as well as for auricles preincubated for 30 min with propranolol (10^{-7} M), indomethacin (1.2×10^{-5} M) or acetylsalicylic acid (1.8×10^{-4} M). Tests were also carried out on preparations from chemically sympathectomized animals injected 24 h prior to sacrifice with 16.5 mg kg^{-1} of 6-OHDA[16]. In order to assess the adequacy of denervation, the in vitro influences of tyramine and norepinephrine (NE) were explored on preparations obtained from animals injected with 6-OHDA. TXB_2 was provided by Upjohn Laboratories. Other drugs were purchased from standard commercial sources. Experimental records were compared with initial controls and expressed as percent changes. Differences between mean values were considered significant if $p = 0.05$ or less.

Results. As shown in figure 1, TXB_2 (10^{-8} M-10^{-5} M) produced a dose-dependent increment of the IDT (a) and of the contractile frequency (b) of spontaneously beating isolated rat atria. The maximal effect of each concentration was observed 2-4 min following the delivery of the agent. Figure 1 also depicts the significant inhibition of the positive inotropic (a) and chronotropic (b) effects of TXB_2 observed in preparations exposed to propranolol (10^{-7} M) as well as in those obtained from animals subjected to chemical sympathectomy with 6-OHDA. The latter auricles

Absolute magnitude of isometric developed tension (IDT) and contractile frequency of rat auricles. Effects of (−)-propranolol, 6-hydroxydopamine (6-OHDA), indomethacin and acetylsalicylic acid (ASA)

Condition	IDT (mg)[a]	Frequency (beats/min)[a]	(n)
Untreated[b] (controls)	430.4±20.2	157.1± 9.6	9
(−)-Propranolol[c] (1.0×10^{-7} M)	412.6±26.2	152.6± 7.6	6
6-OHDA[b] (16.5 mg·kg^{-1}, i.v.)	442.7±30.9	148.3±10.6	6
Indomethacin[c] (1.2×10^{-5} M)	436.2±25.1	155.2± 8.3	7
ASA[c] (1.8×10^{-4} M)	426.3±19.3	149.8± 8.2	6

[a] Means±SEM. [b] Initial values recorded at 10 min following equilibrium (see 'methods'). [c] Values existing at 30 min after addition.

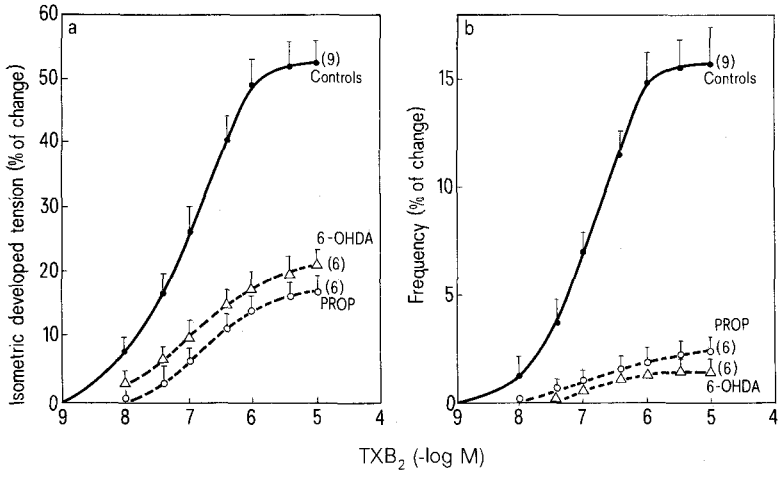

Fig. 1. Cumulative dose-response curves of TXB_2 in spontaneously beating isolated rat atria. Influences of propranolol (PROP) and 6-hydroxydopamine (6-OHDA). IDT isometric developed tension. Points and bars represent the mean and the SEM, respectively. Figures in parentheses indicate the number of preparations.

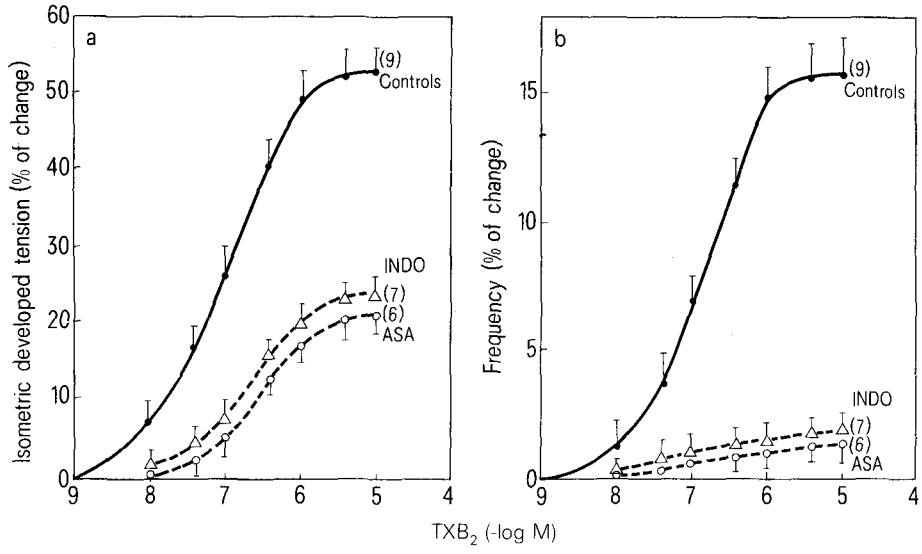

Fig. 2. Cumulative dose-response curves of TXB_2 in spontaneously beating isolated rat atria. Influences of indomethacin (INDO) and acetylsalicylic acid (ASA). For further explanations see legend of Fig. 1.

were hypersensitive to 10^{-8} M NE and refractory to 10^{-6} M tyramine.

A preincubation, with indomethacin (1.2×10^{-5} M) or ASA (1.8×10^{-4} M) also inhibited significantly the inotropic (fig. 2 a) and chronotropic (fig. 2 b) responses to TXB_2. It is noteworthy that the absolute values of IDT and frequency of control atria as well as of those exposed to (−)-propranolol, indomethacin or ASA were comparable before the addition of TXB_2 (table).

The contractile peak tension of the left auricles driven at a constant frequency of 3.3 Hz was also stimulated by 10^{-6} M TXB_2 ($+62\% \pm 8.2$; $n = 5$. Mean ± SEM).

Discussion. The present report demonstrates that TXB_2 increases inotropism and chronotropism of isolated rat atria. The TXB_2-evoked increment of atrial tension cannot be considered as an effect secondary to the enhancement in frequency. Indeed, it is known that rat atria present a negative staircase[13,17,18], and in addition preparations paced at a fixed rate were stimulated similarly to those beating spontaneously. The fact that both actions of TXB_2 were inhibited by beta adrenoceptor blockade as well as after a pretreatment of the animals with 6-OHDA suggests that the effect of TXB_2 is indirect i.e. via a mechanism involving the release of neuronal catecholamines followed by an activation of beta-adrenoceptors.

There are recent observations indicating that TXB_2 can reduce the contractile force of the isolated perfused rat heart, without affecting the beating rate[11]. Since this decline of the tension was accompanied by an elevation of coronary perfusion pressure, it is plausible that the reported mechanical decrement following TXB_2 may be associated with alterations of the coronary pressure. The fact that inhibitors of endogenous prostaglandin synthesis, such as indomethacin or ASA, attenuated the stimulating effect of TXB_2 on the inotropism and chronotropism of rat atria suggests that the compound may be acting through the generation of other prostaglandins. It is also possible that the antagonistic action of indomethacin could result from its influence on c-AMP dependent protein kinases affecting in turn phosphorylation processes involved in the influx of Ca^{2+} [19]. However, the possible participation of other PGs is supported by our recent finding documenting that PGI_2 increased frequency and tension of isolated rat atria[20]. In addition, the augmentation of contractions following PGI_2 is likely to be the result of an indirect catecholamine-mediated mechanism[15,20]. Therefore it is plausible that TXB_2 enhanced rat atrial tension and frequency, increasing first the synthesis and release of prostacyclin, which secondarily augments the release of neuronal noradrenaline with the subsequent stimulation of beta-adrenoceptors in the atrial cells.

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