The Effect of Trimepranol® on Thrombocyte Function and Histamine Release in the Rat

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Abstract

Trimepranol® a beta-adrenergic blocking drug released serotonin from rat isolated and plasma-rich thrombocytes in vitro. The release was time- and dose-dependent. With the same concentrations this drug inhibited the histamine release from isolated rat mast cells. Aggregation of isolated or plasma-rich thrombocytes induced by ADP was inhibited by Trimepranol®. The inhibition was dose, time dependent and reversed by calcium ions.

Introduction

Thrombocytes and mast cells seem to play an important role in inflammatory reactions. Blood platelets contain serotonin (5-HT) and are involved in blood-clotting reaction. Major storage sites for histamine and heparin are in the mast cells. Both are released in the process of degranulation [5, 17].

Histamine is liberated from mast cells in the first period of inflammation [11], serotonin may play permissive or supportive role in the maintenance of chronic inflammatory edema together with prostaglandins [6, 27].

Many drugs, among them beta-adrenergic blocking agents stabilize cell membranes by non-specific mechanism [12]. Moreover, these drugs in low concentration block the uptake of 5-HT into blood platelets, in higher concentration release labeled 5-HT from these cells [13].

The effect of Trimepranol® (TMP) a beta-adrenergic blocking drug was studied on the release of serotonin from thrombocytes and histamine from mast cells. Release studies were compared with the effect of this drug on platelet aggregation.

Materials and methods

Trimepranol® Spofa 1-(2, 3, 5-trimethyl-4-acetoxy-phenoxy)-3-isopropylamino-2-propanol tartarate was kindly supplied by Dr Trčka, VÚFB, Praha.


All other materials were obtained from normal commercial sources.

In all experiments male 300–350 g Wistar rats were used. In ether anesthesia a polyethylene catheter was inserted into common carotid artery and blood was collected in the plastic tubes containing anticoagulant (see below) in a ratio 1:10 (1 ml anticoagulant and 9 ml of blood).

For aggregation studies heparin-citrate mixture (25 IU heparin per 1 ml of blood in 3.8% Na-citrate) was used, for 5-HT release studies chelating mixture (120 mM NaCl and 2.7 mM Na2EDTA) was taken.

Thrombocyte aggregation studies. Blood from 4 animals in each experiment was centrifuged 300 g for 20 minutes at 4°C to get platelet rich plasma (PRP). Isolated thrombocytes were obtained after following centrifugation of PRP 700 g for 15 minutes at 4°C and washed once in calcium-free Tyrode solution (130 mM NaCl, 5.6 mM KCl, 1.0 mM NaH2PO4 x H2O, 25 mM NaHCO3, 11.1 mM glucose, 13.1 mM saccharose) by means of centrifugation (600 g, 10 minutes, 4°C). After resuspension the pellet of platelets in calcium-free Tyrode, thrombocytes were pooled and diluted to final volume to get approximately 700,000–800,000 cells per 1 mm² as counted by the method of FEISSLY and LUDIN [4].

The aggregation was measured both for PRP or isolated thrombocytes by BORN's method [3] in celulose-nitrate tubes photometrically at 620 nm. Suspension was mixed with plastic-coated magnet at 1000 rpm/min. The change in extinction of the photometer was concomitantly recorded on the Beckman linear recorder.

Serotonin release studies. Isolated thrombocytes were obtained from Na2EDTA blood by means of differential centrifugation (see above), washed once in calcium-free Tyrode with Na2EDTA (0.8 g per 1000 ml). Pooled thrombocytes from 4 rats (850,000 cells per 1 mm² in 2 ml sample) were incubated with TMP under different ex-
Effect of Trimepranol® on Thrombocyte Function

Experimental conditions (see results) in plastic tubes. After incubation at 37°C the tubes were cooled to 0°C and centrifuged 700 g for 8 minutes at 4°C. The supernatant was decanted and the sediment resuspended in 2 ml of 0.02 N HCl. Serotonin was determined spectrofluorometrically both in supernatant and sediment (broken cells) by the method of Weissbach and Redfield [24], after precipitation with ZnSO₄ and NaOH. The percentage of the released 5-HT into the supernatant was calculated from the total amount.

If PRP was used, the incubation and centrifugation procedures were the same. The 5-HT in the sediment (thrombocytes) was determined as above [24], in the supernatant by extraction into butanol-heptan by the method of Strauss et al. [21] and Maickel et al. [14].

Studies on mast cells. Mast cells were isolated from pleural and peritoneal washes by the method of Thon and Uvnäs [22]. Separation of mast cells was done by differential centrifugation in Ficoll and after two washes in buffered salt solution (154 mM NaCl, 2.7 mM KCl and 0.9 mM CaCl₂) containing 10 per cent v/v Sörensen phosphate buffer pH 7 and 1 mg human serum albumin (Imuna S. Michal'any). After the isolation, mast cells were pooled and used in amount 150,000 per sample (2 ml). The incubation at 37°C with TMP and compound 48/80 was terminated by cooling the tubes to 0°C and the samples were centrifuged 450 g for 10 minutes at 4°C. The supernatant was decanted and sediment resuspended in 0.01 N HCl, than heated for 6 minutes at 80°C to release all the histamine left. The histamine was determined both in supernatant and sediment spectrofluorometrically by the method of Store et al. [20]. The extraction procedure was omitted according to Bergendorff and Uvnäs [2]. Histamine release in supernatant was evaluated as a percentage of the total histamine content. In all experiments double samples were taken. Each point of the curves represents the mean from 5 to 8 experiments ± S.D. Results were evaluated statistically by Student’s t-test.

The molarity of Trimepranol® in all experiments is expressed as the final concentration in the sample.

Results

Serotonin release from thrombocytes after TMP

Figure 1 shows the release of endogenous 5-HT from isolated rat thrombocytes at 37°C in the presence of TMP. The concentration 10⁻³ M released about 20% of the total amount in the first minute, after one hour almost all 5-HT was released. The TMP in concentrations 10⁻⁴ M and 10⁻⁵ M released after 60 minutes 50 and 30% 5-HT respectively. From the release curves the time necessary for 50 or 25% release (ET₅₀ or ET₂₅) could be evaluated.

The dose-response curve for 5-HT release at different time intervals (5, 15, 30 and 60 minutes) is demonstrated on Figure 2. The relation between the logarithm of increased dose of TMP and the amount of 5-HT released was linear after 30 minutes of incubation. This relationship was pronounced after 60 minutes of incubation.

Serotonin release from PRP after TMP

If the PRP was incubated with TMP in concentration 10⁻³ M (Fig. 3) there was a release of 4.3 ± 0.9% in the first minute and 6.9 ± 1.1% release after 5 minutes. Both values are identical with spontaneous 5-HT release. Release of 16.8 ± 2.1% after 15 minutes and 27.6 ± 1.9% after 30 minutes are significantly higher than the control values.

The effect of TMP on histamine release from isolated mast cells

Figure 4 shows the effect of TMP in concentrations from 10⁻⁵ to 10⁻³ M on the histamine release from isolated rat mast cells. The release was 6.8 ± 0.8% after 10⁻⁵ M, 7.1 ± 1.1% after 10⁻⁴ M and 8.0 ± 0.9% after 10⁻³ M. Spontaneous release was 6.5 ± 0.5%.