

PROPRANOLOL PREVENTS THE DEVELOPMENT OF VENOUS THROMBOSIS IN RATS BY A PLATELET-DEPENDENT MECHANISM

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Propranolol prevents the development of venous thrombosis in rats by a platelet-dependent mechanism. M. GRUSZECKI, R. RÓLKOWSKI, R. PAWLAK, W. BUCZKO. Pol. J. Pharmacol., 2001, 53, 5–10.

To clarify if one of the most common antihypertensive drugs, propranolol, can prevent venous thrombotic process, rats were treated with propranolol (PRO; 5 mg/kg *ip*) in an acute or chronic (14 days) manner. Both regimens resulted in a marked reduction of the systolic blood pressure ($p < 0.001$) and, probably as a consequence, in the shortening of the bleeding time ($p < 0.01$). After ligation of the *vena cava*, the incidence of the venous thrombosis and the thrombus weight decreased significantly in both propranolol-treated groups ($p < 0.01$) when compared to control rats. The anti-thrombotic effect of PRO was not accompanied by any changes in activated partial thromboplastin time, prothrombin time or euglobulin clot lysis time. However, long-term administration of PRO resulted in a reduction of the ADP-induced platelet aggregation.

Key words: *propranolol, venous thrombosis, platelet aggregation, rat*

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INTRODUCTION

Propranolol is one of the most commonly used antihypertensive drugs. It is not entirely clear whether the antihypertensive effect of this drug results solely from the blockade of central and peripheral beta-adrenergic receptors with concomitant suppression of the renin-angiotensin system. There is increasing evidence that this mechanism may be more complex [19]. Such hypothesis has been validated by the recent finding that another beta-blocker, sotalol, can liberate hypotensive autacoids such as nitric oxide and prostacyclin from the endothelium [11], which markedly contribute to its hypotensive action.

Moreover, it is becoming more and more clear that, beside the blood pressure lowering effect, beta-blockers may exert additional beneficial actions in particular groups of patients. For instance, sotalol stimulates fibrinolytic activity of the plasma [11], which may help to prevent thrombotic events in patients suffering from coronary heart disease. Anti-thrombotic activity of propranolol seems to be more controversial. On the one hand, propranolol suppresses the ADP- or collagen-stimulated platelet aggregation [10, 20] and decreases the beta-thromboglobuline plasma levels [1], which may suggest its antithrombotic activity. On the other hand, decreased fibrinolytic activity of the plasma following propranolol administration has been described in spontaneously hypertensive patients [21].

Serotonin serves as one of the endocrine factors responsible for the regulation of circulatory homeostasis, including blood coagulation and fibrinolysis [8]. Serotonergic mechanisms are profoundly altered in spontaneously hypertensive patients [3] and rats [18]. Since propranolol has been known to interact with peripheral and central serotonin receptors [13], in this study we aimed to evaluate the influence of propranolol on venous thrombosis in rats, and to check the role of serotonin in this effect.

MATERIALS and METHODS

Animals

Male Wistar rats (200–280 g) were used in this study. The animals were housed in a room with a 12 h light/dark cycle, in group cages as appropriate, were given tap water and fed a standard rat chow. Twenty four hours before the induction of

venous thrombosis, the rats were deprived of food but had free access to water. Procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research (Thromb. Haemost. 1987, 58, 1078–1084).

Drugs and reagents

Propranolol (Propranololum hydrochloricum, 1 mg/ml, Polfa, Poland), pentobarbital (Vetbutal, Polfa, Poland), ADP (Sigma, USA), serotonin (5-hydroxytryptamine hydrochloride, Sigma, USA), 3.13% trisodium citrate (Polish Chemical Reagents, Gliwice, Poland) were used in the experiments.

Venous thrombosis, bleeding time and blood pressure

The animals received propranolol either in a single dose (5 mg/kg) or for 14 days (the same dosage, once daily) by intraperitoneal (*ip*) injection. The rats injected with isotonic saline (0.9% NaCl) served as controls. In these conscious animals, “template” bleeding time was measured by the standardized tail incision according to Dejana et al. [9]. The mean arterial blood pressure (the “tail cuff” method, Student Oscillograph, Harvard Rat Tail Blood Pressure Monitor) was estimated in conscious animals [22], and in anesthetized rats 5 min before and 100 min after the induction of venous thrombosis. One hour after the last administration of propranolol (or isotonic saline) the animals were anesthetized with pentobarbital (45 mg/kg *ip*) and venous thrombosis was performed as previously described by others [17]. Briefly, the abdomen was opened, the *vena cava* was carefully separated from the surrounding tissues and then ligated tightly with a cotton thread just below the left renal vein. Subsequently, the abdomen was closed with a double layer of sutures (peritoneum with muscles and the skin separately). After 2 h the abdomen was reopened, the *vena cava* was carefully dissected and inspected for the presence of a thrombus. The thrombus was kept at 37°C and after 24 h its dry weight was measured.

Platelet aggregation

Blood samples from rats treated with 5 mg/kg of propranolol for two weeks and obtained from drug-naive animals were taken from the heart un-

der pentobarbital (45 mg/kg *ip*) anesthesia, and mixed with 3.13% trisodium citrate in a volume ratio 9:1. The platelet-rich plasma (PRP) was obtained by centrifugation of the blood at $450 \times g$ for 2 min at room temperature. The platelet count of PRP was adjusted to 300 000 platelets/ μ l by appropriate dilution with autologous platelet-poor plasma (PPP). The assay for blood platelet aggregation was carried out according to Born and Hume [2] using an Elvi 840 aggregometer connected to a recorder OH-81411 (Radelkis, Budapest, Hungary). Samples of 0.25 ml PRP were preincubated at 37°C for 1 min before the addition of an aggregating stimulus (4 mM ADP; 10^{-8} M serotonin, in a total volume of 10 μ l). The results are presented as the percentage of the light transmission in relation to the control (i.e. the aggregation of platelets obtained from propranolol-naive animals).

Hemostatic parameters

Blood samples were carefully taken from the heart to assess hemostatic changes in systemic circulation. All samples were mixed with 3.13% trisodium citrate in a volume ratio 9:1. PPP was obtained by centrifugation of the blood at $450 \times g$ for 20 min at 4°C. Samples of 0.15 ml were immediately frozen at -20°C. Then prothrombin time (PT) and activated partial thromboplastin time (APTT) were automatically determined by optical method (Coag-A-Mate XM, Organon Teknika, Belgium) using routine laboratory reagents (Organon Teknika, Belgium), and euglobulin clot lysis time (ECLT) was determined according to Lidbury et al. [12].

Statistical analysis

The data are shown as means \pm SEM. In calculating the thrombus weight, lack of a thrombus was regarded as 0 mg. The blood pressure, the thrombus weight and platelet aggregation were evaluated using a one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls Multiple Comparisons Test. The incidence of venous thrombosis was compared by means of the exact Fisher test. The *p* values less than 0.05 were considered significant.

RESULTS

Since all the parameters measured in both control groups (i.e. given isotonic saline once or for two weeks) did not differ, these groups were put together for the purpose of statistical comparison.

The incidence of venous thrombosis decreased to 75% and to 61% in the group of animals administered with propranolol acutely or for two weeks, respectively (in comparison with control rats: 91%, see Fig. 1A).

In control rats, the dry thrombus weight amounted to 2.6 ± 0.14 mg. Acute or chronic propranolol administration resulted in a marked decrease in this parameter to 0.9 ± 0.07 (*p* < 0.01) and 0.7 ± 0.05 (*p* < 0.01), respectively (Fig. 1B).

Systolic blood pressure measured by the “tail cuff” method in conscious rats amounted to 136 ± 3 mmHg in the control group, and was reduced by propranolol administered acutely (116 ± 3 ; *p* < 0.001) or for two weeks (116 ± 3 ; *p* < 0.001). Similar results were obtained after *vena cava* ligation, although the ligation itself markedly reduced systolic blood pressure (from 111 ± 5 to 86 ± 4 ; *p* < 0.001).

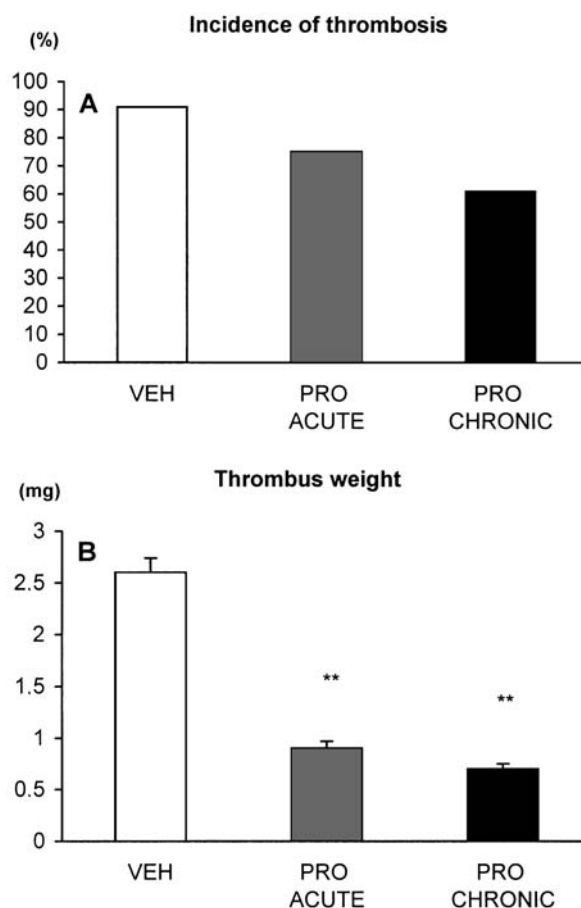


Fig. 1. Columns represent (A) the incidence of thrombus formation and (B) the thrombus weight in rats treated with propranolol (5 mg/kg *ip*) in acute (PRO ACUTE; *n* = 12) or chronic (14 days; PRO CHRONIC; *n* = 12) manner. ** *p* < 0.01 vs control (VEH; *n* = 18). Data are expressed as means \pm SEM

In control rats the “template” bleeding time amounted to 107 ± 3 s. Acute or chronic propranolol administration resulted in a marked decrease in this parameter to 73 ± 3 ($p < 0.01$) and 63 ± 2 ($p < 0.01$), respectively (Fig. 2).

ADP-induced platelet aggregation was suppressed by chronic propranolol administration by 44%, comparing to the control, drug-naive animals

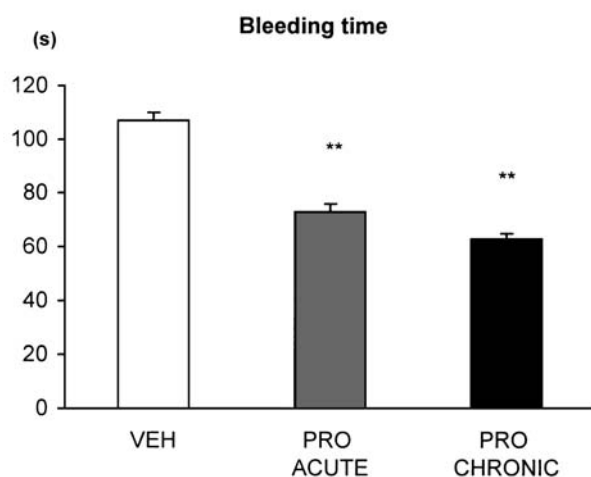


Fig. 2. Columns represent the “transection” bleeding time in rats treated with propranolol (5 mg/kg *ip*) in acute (PRO ACUTE; $n = 9$; 32% reduction) or chronic (14 days; PRO CHRONIC; $n = 7$; 41% reduction) manner in comparison with the control (VEH). ** $p < 0.01$ vs control (VEH; $n = 7$). Data are expressed as means \pm SEM

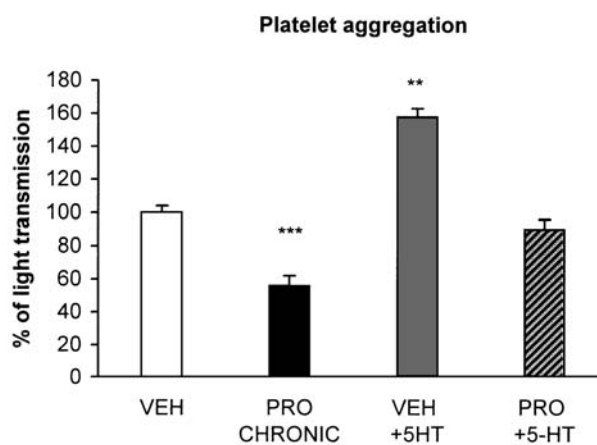


Fig. 3. Columns represent the ADP-induced platelet aggregation (with or without potentiation by serotonin) in rats treated with propranolol (5 mg/kg *ip*) in chronic (14 days; PRO CHRONIC; $n = 10$) manner in comparison with the control (VEH; $n = 8$). ** $p < 0.01$; *** $p < 0.001$ vs control (VEH). Data are expressed as means \pm SEM

($p < 0.01$; Fig. 3). Similar, but less pronounced suppression was observed with respect to the serotonin-induced augmentation of ADP-induced platelet aggregation (Fig. 3).

No differences in euglobulin clot lysis time, prothrombin time or activated partial thromboplastin time were observed in the above-described groups of animals (Tab. 1).

Table 1. Activated partial thromboplastin time (APTT), prothrombin time (PT) and euglobulin clot lysis time (ECLT) in rats treated with propranolol (5 mg/kg *ip*) in acute (PRO ACUTE; $n = 5-9$) or chronic (14 days; PRO CHRONIC; $n = 12-16$) manner in comparison with the control (VEH; $n = 6-11$). Data are expressed as means \pm SEM

	APTT (s)	PT (s)	ECLT (min)
VEH	24.4 \pm 2.0	27.5 \pm 1.5	215 \pm 33
PRO ACUTE	21.3 \pm 1.1	28.4 \pm 1.4	139 \pm 25
PRO CHRONIC	20.1 \pm 0.8	28.3 \pm 1.1	217 \pm 29

DISCUSSION

In this study, we used a well established model of venous thrombosis in rats [6, 7, 14, 15, 17]. The conditions of thrombus formation in this experimental design closely resemble the venous thrombosis development in humans. Thus, this model is sensitive to the typical hemostatic alterations that may affect the thrombus development in man.

The major finding of this study is that propranolol, after its acute or chronic administration, slightly diminished the incidence of thrombosis and markedly reduced the thrombus weight in normotensive rats. Thus, we demonstrated that this beta-blocker could exert antithrombotic effect in venous thrombosis model. The mechanism of such an action is not clear. Undoubtedly, several possibilities must be taken into consideration. One of the most obvious is the effect on the blood coagulation. To check if this effect was caused by the influence on blood coagulation system we estimated the indices of intrinsic and extrinsic pathways of coagulation cascade. We have shown that antithrombotic effect of propranolol was independent of the changes in prothrombin time and activated partial thromboplastin time, thereby excluding such possibility.

It has been shown that other beta-blockers, sotalol for example, can augment fibrinolysis by NO/PGI₂-dependent liberation of tissue-type plas-

minogen activator [11]. Thus, in the next step of our study, by estimating euglobulin clot lysis time, we measured fibrinolysis following propranolol administration. However, we could not detect any changes in this parameter in propranolol-treated rats, neither after acute nor chronic administration of the drug, excluding thereby the augmentation of fibrinolysis as a cause of beneficial effect of propranolol.

Another possible mechanism of the antithrombotic effect of the studied beta-blocker is the suppression of the activity of blood platelets. In spite of the common belief that the role of platelets in venous thrombotic process is negligible [17], the ability of classical antiplatelet agents to prevent the thrombus formation inside the pathologically altered veins has been recently demonstrated [16]. Thus, in the next step of our study, we checked if the antithrombotic action of propranolol could be attributed to the suppression of the activity of thrombocytes. Indeed, we have shown the ability of propranolol to reduce platelet aggregability. This result is in line with previous studies demonstrating antiplatelet effect of propranolol after stimulation with various agonists [10, 20]. It is tempting to speculate, therefore, that this action of propranolol could be attributed to its membrane stabilizing effect which may result in the decrease in the platelet aggregatory threshold.

Therefore, we consequently checked if propranolol could alter the interaction of platelets with the vessel wall. Typically, if the activity of platelets is reduced, the bleeding time should be prolonged [9]. In our study, however, propranolol reduced the bleeding time, both after acute and chronic administration. This parameter, albeit commonly used to assess the platelet/vessel wall interaction, is a very rough in *in vivo* method, which can be biased by numerous factors, including blood pressure. Therefore, one plausible explanation of such an effect of propranolol on the bleeding time could be a blood pressure reduction. Thus, we measured blood pressure after administration of propranolol and found a marked decrease in this parameter, which could be responsible for the shortening of the bleeding time. However, it is unlikely for the blood pressure reduction to be responsible for the beneficial effect of propranolol on the venous thrombosis. As we have demonstrated in our previous studies, there is no correlation between blood pressure and the venous thrombus weight reduction in the case of

other hypotensive drugs, such as angiotensin-converting enzyme inhibitors [4, 5, 14, 15] or AT₁ angiotensin receptor antagonists [5–7].

In summary, we have demonstrated that propranolol exerted an antithrombotic effect in a venous thrombosis model in normotensive rats, both after acute and chronic administration of the drug, which suggests the involvement of some fast-acting mechanism(s). This effect was accompanied by the suppression of the platelet aggregation, but neither the changes in the extrinsic or intrinsic pathways of the coagulation cascade nor the increase in fibrinolysis were observed. Propranolol reduced the bleeding time, the effect that can possibly be attributed to its blood pressure lowering effect.

Acknowledgment. This work was supported by Grant No. 3-11827 from the Medical Academy of Białystok, Poland.

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Received: December 20, 2000; in revised form: February 7, 2001.