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THROMBOLYTIC ACTIVITY OF β-ADRENOLYTIC DRUG, SOTALOL

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Sotalol is a β-adrenoreceptor blocking drug, the clinical efficacy of which has been linked up to its negative chrono- and inotropic effects and its hypotensive action. In addition, β-adrenolytic drugs are known to inhibit platelet aggregation *in vitro* possibly through lowering of calcium ions level. Here, we report that in rats sotalol at a dose of 10—20 mg/kg i.v., apart from hypotension, evokes instantaneous thrombolytic effect. This is associated with an increase in plasma level of tissue plasminogen activator (t-PA). *In vitro*, sotalol at a concentration of 1—100 μM inhibits thrombogenesis on surface of rabbit aorta endothelium superfused with blood. Sotalol also has a weak anti-aggregatory activity ($IC_{50} \sim 500-1000$ μM) in human platelet rich plasma (PRP). Since the thrombolytic and fibrinolytic but not hypotensive effects of sotalol were inhibited by cyclooxygenase inhibitor, indomethacin, while its hypotensive but not thrombolytic potency was dimished by an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine (L-NNA), we have linked up the sotalol-induced effects *in vivo* with the release of prostacyclin and nitric oxide. Our data point out to a possibility that prostacyclin and nitric oxide concomitantly released from endothelium and/or from other blood cells after administration of sotalol, may play different roles: prostacyclin may be responsible for fibrinolytic, thrombolytic and antithrombotic properties, while nitric oxide may take part in the mechanism of sotalol-induced hypotension.

Key words: *sotalol, β-adrenoreceptor blocking drugs, prostacyclin (PGI_2), nitric oxide (NO), thrombolysis, fibrynolysis, thromboresistance, evglobulin clot lysis time (ECLT), N^G-nitro-L-arginine (L-NNA)*

INTRODUCTION

β-adrenolytic drugs, apart from being useful in the treatment of hypertension, coronary insufficiency and dysrythmias, are important means of preventing the primary and secondary myocardial infarction (1). Recent clinical trials indicate that these drugs lower the mortality rate after myocardial infarction, and decrease the frequency of the secondary myocardial infarction (1). So far, it is commonly accepted that therapeutic efficiency of β-adrenolytic drugs results from a decrease in heart rate, contraction force of cardiac muscle and lowering of blood pressure. It is claimed that β-adrenolytic drugs inhibit also thrombin- and calcium ionophore A23187-induced platelet aggregation

(2, 3). We have demonstrated that administration of β-adrenolytic drug both into animals and humans (4, 5), is associated with activation of thrombolysis and the mechanism of such activity may consist in enhancement of the release of endogenous prostacyclin (PGI_2) as a result of switching off the tension at the adrenergic receptors. In the mechanism of release of thrombolytic prostanoids the adrenergic system seems to constitute natural counterbalance for cholinergic system and this is why the release of prostanoids may possibly appear both as a result of blocking of β-adrenergic receptors and/or following the activation of parasympathetic system with cholinergic agonist that we observed some years ago (5).

Recently, some progress has been made in investigation of the secretory function of vascular endothelium and its role in the activation of thrombolysis. It was found that the release of thrombolytic prostacyclin would be accompanied by the release of nitric oxide (NO) (6, 7, 8). Both mediators are potent antiplatelet and vasodilating agents, although the mechanisms of their action are different. PGI_2 acts through activation of adenylate cyclase, whereas NO acts through activation of guanylate cyclase (9, 10). At the same time both compounds were found to activate thrombolysis (11, 12), and while the thrombolytic activity of prostacyclin seems to be due to the release of tissue plasminogen activator (t-PA; 13), the same activity of NO is possibly due to inhibition of the release of plasminogen activator inhibitor (PAI; 11). Thus, it is tempting to speculate that β-adrenolytic drugs can affect plasma fibrinolytic system by interfering with the release of PGI_2 and NO. It is also possible that β-adrenolytic drugs, especially those with strong membrane-stabilizing properties, may affect the thrombolytic system directly *via* inhibition of the release of PAI from blood platelets. This is based on the fact that inhibition of platelet aggregation, observed for β-adrenoreceptor blocking drugs (3), is common for all known inhibitors of PAI release from platelets (12).

Here, we investigate the influence of sotalol on functioning of fibrinolytic system in animal models *in vivo*, *ex vivo* and *in vitro*, taking into account the direct action of sotalol as well as its effects on mediating of the release of PGI_2 and NO. It is the first attempt to elucidate the mechanism of antithrombotic effect of one of the β-adrenoreceptor blocking drugs — sotalol.

METHODS

Thrombolysis in vivo

Evaluation of thrombolytic activity of sotalol was performed *in vivo* in anaesthetized rats with extracorporeal circulation (14). Briefly, animals weighing from 400 to 500 g were anaesthetized with thiopental (95 mg/kg i.p.) and heparinized (800 U/kg i.v.). For monitoring of the mean arterial pressure the Isotec type electronic transducer was connected with a cannula to the left carotid artery, whereas the cannulated right carotid artery was delivering blood into the extracorporeal

circulation by means of a peristaltic pump (37°C, 1ml/min). The blood withdrawn superfused the isolated collagen strip. Following superfusion of the collagen strip the blood returns into the circulation through the cannulated left femoral vein. Weight of the superfused strip is continuously monitored by means of the modifed 386 Harvard transducer. During superfusion a clot is formed on the surface of the collagen strip. As confirmed by the electron microscopy the clot consists of platelet aggregates that are connected with each other by fibrin threads, with single neutrophils or erythrocytes trapped here and there. Formation of the clot results in gain of weight proportional to the size of the clot, and after 20—30 min reaches plateau. We proved (15, 16) that exogenous prostacyclin and releasers of endogenous prostacyclin (e.g. nicotinate, kinins) would dissipate thrombi and cause loss in weight of the collagen strip.

Following drugs were given intravenously: sotalol (Polfa); 10—30 mg/kg, indomethacin (Sigma); 10 mg/kg, inhibitor of nitric oxide synthase — N^G-nitro-L-arginine (L-NNA, Sigma); 10 mg/kg. Indomethacin or L-NNA was administered 15 min before injection of sotalol.

Assay of endothelial thromboresistance

In vitro interaction between blood and vascular endothelium was studied under flow conditions (17). Briefly, an inverted inside out tubular segment of rabbit aorta, taken from a killed animal, was attached to the auxotonic lever of the Harvard transducer and superfused (2 ml/min) with citrated (3.8%; 1:9 v/v) blood of rabbit (37°C) that was gassed with 95% 0_2 + 5% $C0_2$. Blood for experiments was obtained from previously anaesthetized animals by a heart puncture. A pre-scaled Watanabe recorder was used to monitor formation of thrombi by registering changes in weight of superfused aortas. In this system, maximal gain in weight (expressed in mg of the formed thrombi) at the steady state is inversely proportional to endothelial thromboresistance against adhesion and aggregation of morphological elements of noncoagulating blood. As checked by electron microscopy, the main component of thrombi were platelets whereas fibrin leukocytes and erythrocytes constituted its minor component. In all experiments natural thromboresistance of rabbit aortas was pharmacologically decreased by soaking them in solutions containing aspirin (100 μM) and L-NNA (300 μM), inhibitors of prostacyclin and nitric oxide synthesis, respectively. Thrombogenesis was modulated by sotalol (1—100 μM) added into superfusing blood 20 min before experiment.

Assay of fibrinolysis ex vivo

Fibrinolytic activity of sotalol was assayed in plasma samples of anaesthetized rats 10 min after administration of sotalol (20 mg/kg i.v.) or saline as activity of tissue plasminogen activator (t-PA), using commercial kits from Biopool (Sweden). In some experiments 20 min before administration of sotalol, the animals were pretreated with indomethacin at a dose of 10 mg/kg i.v.

The same plasma samples were used for the assay of euglobulin clot lysis time (ECLT) (18). Blood from carotid artery was withdrawn into trisodium citrate (3.15%) at a ratio of 9:1, then centrifuged at 15000 rpm for 1 min to produce platelet poor plasma (PPP) which at a volume of 0.5 ml was subjected to the assay of t-PA activity according to the kit manual. The rest of PPP was diluted with distilled water (1:14 v/v) and its pH was adjusted to 5.4 by bubbling with CO_2 for 3 min. This procedure leads to precipitation of the euglobulin fraction while acidity destroys the biological activity of plasminogen activator inhibitor (PAI). After a second centrifugation at 15000 rpm for 1 min the supernatant was discarded and the euglobulin precipitate dissolved in 1 ml phosphate buffer. Two units of human thrombin from the stock solution (200 U thrombin/ml 0.05 M $CaCl_2$) was added to 0.3 ml of euglobulin sample to induce formation of the clot. It was incubated at 37°C and time required for complete lysis was recorded.

Assay of the release of tissue plasminogen activator inhibitor (PAI-1) from platelets in vitro

Experiments were performed in 1 ml samples of rabbit platelet rich plasma (PRP) added to plastic tubes and incubated without (saline) or with sotalol (1000 μM) for 30 min at 37°C with a gentle agitation. At time 0 and after 30 min of incubation the level of PAI released from platelets was evaluated spectrophotometrically, using commercial kits from Biopool (Sweden) according to the procedure described elsewhere (12).

Platelet aggregation in vitro

Platelet aggregation was measured in a Born aggregometer in human platelet-rich plasma (PRP) stimulated with collagen, adrenaline and ADP in the presence or absence of sotalol.

Aggregation in PRP was performed according to the Born's method in a Chronolog optic aggregometer with constant stirring at 37°C. The system measures changes of the optical density of platelet suspension in response to aggregating agents. Venous blood was taken from healthy volunteers, who had abstained from taking any drugs for at least 10 days. The blood was collected into 3.8% sodium citrate (9:1, v/v). PRP and platelet poor plasma (PPP) were prepared by centrifugation (184 g, 10 min. and 1660 g, 10 min., respectively). The platelet count was kept constant at 2×10^8 by adjusting a number of platelets using PPP. Pro-aggregatory agents, adenosine diphosphate (ADP), collagen and adrenaline were used at threshold concentrations (2—8 μM, 0.1—1.0 mg/ml and 1.5—3.0 μM, respectively). The inhibitors were incubated with PRP for 2 min prior to the addition of aggregating agents. The inhibitory concentration was determined by calculation of percent of a decrease in height of the aggregation curve compared to the control aggregation curve.

Statistical analysis

Statistical analysis was performed using Student's t-test for paired values. Statistical variability was determined basing on the differences between the groups of arithmetic means ± standard error of the mean (mean ± SEM) for the given "n" of experiments.

RESULTS

Thrombolysis in vivo

Administration of sotalol (10—30 mg/kg i.v.) to the anaesthetized rats evoked *in vivo* an immediate (after 3—4min) dose-dependent thrombolysis (*Table 1*). Thrombolysis was reversible and lasted approximately 20 min. For comparison, prostacyclin at a dose of 0.3 mg/kg i.v. caused reversible 25% thrombolysis (n = 25). Intravenous administration of sotalol dose-dependently decreased mean arterial blood pressure (*Table 1*). Thrombolytic (but not hypotensive) effect in response to sotalol was signifcantly inhibited by administration of indomethacin (10 mg/kg i.v.) given 10—20 min prior to the infusion of sotalol. This inhibition was reversible and after 60—80 min following the infusion of indomethacin, sotalol at a dose of 20 mg/kg i.v. regained its thrombolytic activity. In contrast, inhibitor of nitric oxide synthase, L-NNA (10 mg/kg, i.v.), did not affect the thrombolytic activity of

sotalol, but it irreversibly (at least up to 3—4 hrs) inhibited hypotensive action of the drug in a dose-dependent manner.

Table 1. Influence of indomethacin and L-NNA on thrombolysis and mean arterial blood pressure after intravenous administration of sotalol in rats *in vivo*. Indomethacin and L-NNA were injected 15 min before sotalol. Results are expressed as a mean ± SEM for (n) number of experiments. A „p" value (*) of <0.05 was taken as significant in comparison with the effect of sotalol *per se*.

Treatment (dose i.v.)	Thrombolysis (% of control) (mean ± SEM) for (n) number of experiments	Decrease (↓) or increase (↑) of mean arterial blood pressure (mean mm Hg ± SEM)
Sotalol (10 mg/kg)	4 ± 0.4(4)	↓ 30 ± 9.5
Sotalol (20 mg/kg)	24 ± 4(15)	↓ 34 ± 7.4
Sotalol (30 mg/kg)	30 ± 4 (4)	↓ 40 ± 10
L-NNA (10 mg/kg)	no effect (4)	↑ 26 ± 1
Sotalol (20 mg/kg) after pretreatment with L-NNA	20 ± 5.1 (6)	↓ 5 ± 10*
Indomtehacin (10 mg/kg)	no effect (4)	↑ 33 ± 7
Sotalol (20 mg/kg) after pretreatment with indomethacin	4 ± 0.5(6)*	↓ 28 ± 6

Endothelial thromboresistance in vitro

Sotalol dose-dependently (*Table 2*) inhibited thrombogenesis on the surface of endothelium previously treated with a combination of aspirin and L-NNA.

Table 2. Inhibition of thrombogenenesis by sotalol on the surface of rabbit aorta (RbA) endothelium, as measured under whole-blood flow conditions *in vitro*. In aim to increase natural endothelial tromboresistance RbAs were pretreated with combination of aspirin (100 μM) and L-NNA (300 μM) for 20 min before superfusion with blood. A „p" values of <0.05 were taken as significant in comparison with corresponding controls.

Concentrations of sotalol (μM)	Thrombogenesis in mg (mean ± SEM)	% inhibition of thrombogenesis (mean ± SEM)	Statistical significance (p) for 6 experiments in each group
0	210 ± 18		
1	86 ± 9	59 ± 4.1	$p<0.05$
10	71 ± 9	66 ± 5.2	$p<0.001$
30	63 ± 8	70 ± 6.1	$p<0.05$
100	52 ± 7	75 ± 6.8	$p<0.001$

Fibrinolysis ex vivo

A single intravenous injection of sotalol (20 mg/kg)) to the anaesthetized rats 10 min before blood collection signifcantly shortened the euglobulin clot lysis time (ECLT; 26.0% ± 4.0, n=6, $p<0.05$) as compared with the *placebo* group (saline). In the same group of animals sotalol increased the level of tissue plasminogen activator (t-PA) in plasma from undetectable (controls) to 1.7 i.u./ml ± 0.12 (n=6). Administration of exogenous t-PA (10 mg/kg i.v., n=6) in our experimental set up evoked twofold increase in plasma level of t-PA within first minute after injection lasting for 10—15 min. ECLT was shortened by 100%. Shortening of ECLT along with increased activity of the plasma t-PA level can be also induced by intravenous administration of exogenous prostacyclin or its analogue — iloprost — at a concentration below 1mg/kg (n=3). Sotalol-induced rise of t-PA level was completely inhibited by pretreatment of animals with indomethacin (3 experiments)

The release of tissue plasminogen activator inhibitor (PAI-1) from platelets in vitro

Incubation of platelet rich plasma in vitro at 37°C resulted in a gradual increase in the activity of the tissue plasminogen activator inhibitor (PAI) released from blood platelets, that reached its plateau state after approximately 25—30 min of the incubation (8.0 i.u./ml ± 4.0 of PAI in a control plasma to 17.0 i.u./ml ± 4.0 at 30-th min of the incubation, n=6). This increase was not inhibited by addition of sotalol at a concentration of up to 1000 mM (n=5) into the incubation mixture. On the other hand, when SIN-1, a nitric oxide donor (an active metabolite of molsidomine), was added into the incubation mixture at a concentration of 30 mg/ml (n=4) in the same experimental conditions, the release of PAI-1 from platelets was inhibited by 75%.

Aggregation in PRP

Sotalol turned out to be a weak inhibitor of aggregation of human blood platelets (*Table 3*). IC_{50} for the collagen-induced platelet aggregation was 450 μM, and IC_{50} for both ADP- and adrenaline-induced platelet aggregation was approximately 1000 μM. Agents considered as inhibitors of platelet aggregation usually have their IC_{50} below 100 μM.

Table 3. Anti-aggregatory effect of sotalol measured in human platelet rich plasma (PRP) *in vitro.*

Aggregating agents at treshold concentrations	Concentration of of sotalol (μM)	Inhibition of platelet aggregation (mean% ±SEM) (n)-number of experiments
Colagen	100	0 (4)
	300	26±3 (4)
	1000	66±3.1 (4)
ADP	100	21±3.4 (3)
	300	33±3.8 (3)
	1000	52±2.8 (3)
Adrenaline	100	11±1.5 (5)
	300	29±4.1 (9)
	1000	56±2.2 (4)

However, this weak inhibitory activity of sotalol in adrenaline-induced platelet aggregation, was enhanced by sodium nitroprusside. In three experiments sotalol at a concentration of 300 μM inhibited platelet aggregation induced with adrenaline (2 μM) by 21% ±6, and sodium nitroprusside (1 mM) inhibited it by 40% ±3. A combination of sotalol and sodium nitroprusside at the above concentrations inhibited aggregation by 57% ±3 (n=3).

DISCUSSION

Our studies suggest that sotalol, a β-adrenoreceptor blocking drug, being hypotensive, has also fibrinolytic and thrombolytic activity *in vivo* as well as anti-thrombotic and weak antiplatelet activity *in vitro*. Its thrombolytic activity is accompanied by activation of fibrinolytic system resulting in shortening of the euglobulin clot lysis time and in increase of t-PA activity in plasma, but it is not related to inhibition of PAI-1 release from platelets. Since the thrombolytic effect of sotalol is inhibited by cyclooxygenase inhibitor, indomethacin, and, on the other hand, its hypotensive potency diminished by the nitric oxide synthase inhibitor, L-NNA, it is tempting to link up the sotalol-induced thrombolysis and hypotension with the release of endogenous prostacyclin and nitric oxide from vascular endothelium. Moreover, since in *in vivo* experiments indomethacin does not affect sotalol-induced hypotension, whereas L-NNA does not influence thrombolysis, we are in a position to confirm our earlier hypothesis (19) that prostacyclin and nitric oxide, concomitantly released from the endothelium (7), may play different roles. Prostacyclin may be responsible for fibrinolytic, thrombolytic and antithrombotic properties of the

endothelium, while nitric oxide may dilate blood vessels after diffusion to smooth muscles of the vessels. However, could the mechanism of the activity of sotalol be explained entirely on the basis of the release of both mediators from vascular endothelium? This does not seem to be the case at least in our *in vitro* experiments, where pretreatment of vascular endothelium with aspirin and L-NNA shows no effect on sotalol-induced inhibition of thrombogenesis. In this experimental model, in spite of the fact that the inhibitors of vascular synthesis of prostacyclin and NO have increased *per se* the thrombogenic properties of blood-superfused aortic endothelium by 35% and 20%, respectively, in this way supporting our observation that endothelial prostacyclin and NO are additive in maintaining basic endothelial thromboresistance in our *in vitro* model (20), sotalol increased endothelial thromboresistance possibly by a mechanism independent on the release of prostacyclin and NO from endothelium and an additional unknown mechanism of inhibitory effect of sotalol on thrombogenesis cannot be excluded. Probably, this is not the effect of sotalol on the release of PAI from platelets since we have not been able to see such effect *in vitro* experiments. So far, in our *in vitro* studies we failed, as many others did, to elucidate the mechanism of *in vivo* actions and the discrepancy between the results obtained *in vitro* and *in vivo* has to be explained in favour of the latter, especially, that there is also additional evidence *in vivo* speaking for involvement of prostacyclin in mediation of the activity of sotalol. Namely, it came from our fibrinolysis experiments in which sotalol-induced release of t-PA was inhibited by indomethacin. Prostacyclin is known to activate fibrinolytic system *via* the release of t-PA (21, 13) and this is why inhibition of sotalol-induced release of t-PA by cyclooxygenase inhibitor strongly supports the hypothesis on participation of this endothelial hormon in fibrinolytic activity of sotalol *in vivo*.

The mechanism by which sotalol could activate the release of prostacyclin and NO appears a complex issue. Our previous studies pointed out that adrenergic system constitutes natural counterbalance for the release of thrombolytic prostanoids, especially of prostacyclin, when it is released by cholinomimetics (5). Here we suggest that the release of endothelial prostacyclin may result also from inhibition of adrenergic system by its antagonists. Moreover, taking into consideration the fact that L-NNA inhibits hypotensive activity of sotalol, the property ascribed to nitric oxide (22, 23), also involvement of NO in the mechanism of hypotensive action of sotalol seems to be likely.

It should be realized that the endothelium is not the only site being under autonomic regulation that generates antithrombotic prostacyclin and NO *in vivo*. It has become increasingly recognized that adrenergic and dopamine receptors present on lymphocytes and macrophages provide the channels for

noradrenergic signaling to these immune cellls (24) which constitute another source of prostacyclin and nitric oxide *in vivo*. Catecholamines have a wide range of direct effects on immune cells, particularly on macrophages and lymphocytes. Acting, for example, through β-adrenergic and dopaminergic receptors which are linked up to adenylate cyclase, they may modulate lymphocyte proliferation and production of proinflammatory cytokines (24). All the above indicate that catecholamines as well as their antagonists, *via* modulation of the level of c-AMP, may have a potent activity on eicosanoid and NO releasing properties of different blood cells which, as yet, have not been adequately explored. Such assumptions, however, are rather speculative and remain beyond the scope of this paper. Dosage of β-blockers could be essential, at least for their thrombolytic action, as in low doses some of them may exert thrombolytic activity on its own, whereas in high doses the inhibition of prostacyclin release may appear (4).

In summary, sotalol shows hypotensive, thrombolytic and antithrombotic properties, increases endothelial thromboresistance and activates the fibrinolytic system. Our data point out to a possibility that these actions are mediated by the release of endogenous prostacyclin and nitric oxide. Prostacyclin and nitric oxide, concomitantly released from the endothelium (7), may play different roles: prostacyclin may be responsible for fibrinolytic, thrombolytic and antithrombotic properties of the endothelium, while nitric oxide may dilate blood vessels after diffusion to the smooth muscles of the vessels. The role of vascular endothelium as only source of the sotalol-induced release of prostacyclin and nitric oxide is dubious and participation of different blood cells being under adrenergic regulation cannot be excluded in this mechanism. Although a signifcant part of benefits of the therapy with sotalol may be related to the thrombolytic and fibrinolytic properties of endogenous prostacyclin, its rather weak activity and in consequence the need for high dosage of sotalol to induce thrombolysis may limit the clinical applicability of this drug in thrombotic disorders.

REFERENCES

1. Beurrier D, Danchin N. Treatment after myocardial infarction. *Presse Med* 1994; 23: 380—384.
2. Nosal R, Petrikova M, Jancinova V. Alterations in lipid peroxidation of thrombin-stimulated rat platelets treated with beta-adrenoceptor blocking drugs. *J Lipid Mediat* 1993; 8: 121—132.
3. Nosal R, Janchinova V, Petrikova M. The role of intracellular calcium in A23187 stimulated and β-adrenoreceptor blocking drug treated platelets. *Biochem Pharmacol* 1994; 47: 2207—2211.
4. Gryglewski RJ. Mechanisms regulating the release of deaggregating prostanoids. *Kardiol-Pol* 1982; 25 (10—11): 801—812.
5. Święs J, Radomski M, Dembińska-Kieć A *et al.* Stimulatory cholinergic effect on the release of antiaggregatory activity into the circulation of cat and man and its modifcation by β-adrenergic antagonists. *Europ J Clin* Invest 1985; 15, 320—326.

6. Furchgott RF., Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288: 373—376.
7. Gryglewski RJ, Moncada S, Palmer RM. Bioassay of prostacyclin and endothelium-derived relaxing factor (EDRF) from porcine aortic endothelial cells. *Br J Pharmacol* 1986; 87: 685—694.
8. De Nucci G, Gryglewski RJ, Warner TD, Vane JR. Receptor-mediated release of endothelium-derived relaxing factor (EDRF) and prostacyclin from bovine aortic endothelial cells is coupled. *Proc Natl Acad* Sci USA 1988; 85, 2234—2338.
9. Katsuki S, Arnold W, Mittal C, Murad F. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J Cyclic Nucl Res* 1977; 3: 23—35.
10. Rapoport RM, Draznin MB, Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* 1983; 306 (5939):174—176.
11. Korbut R, Lidbury PS, Vane JR. Prolongation of fibrinolytic activity of tissue plasminogen activator by nitrovasodilators. *Lancet* 1990; 335, 669.
12. Lidbury PS, Korbut R, Vane JR. Sodium nitroprusside modulates the fibrinolytic system in the rabbit. *Br J Pharmacol* 1990;101: 527—530.
13. Moore PK, Hussani I, Bhardwaj R. Prostanoids stimulate fibrinolysis in the rat. *Arch Int Pharmacodyn* 1988; 296: 155—162.
14. Gryglewski RJ, Korbut R, Ocetkiewicz A, Stachura J. *In vivo* method for quantitation of antiplatelet potency of drugs. *Naunyn Schmiedeberg's Arch Pharmacol* 1978; 302: 25—30.
15. Gryglewski RJ. Interactions between endothelial secretagogues. *Ann Med* 1995; 27: 421—427.
16. Korbut R, Moncada S. Prostacyclin (PGI_2) and thromboxane A_2 interaction *in vivo*. *Thromb Res* 1978; 13: 489—500.
17. Korbut R, Ocetkiewicz A, Gryglewski RJ. Nitric oxide complements prostacyclin in the regulation of endothelial thromboresistance under flow conditions. *Methods Find Exp Clin Pharmacol* 1993; 15: 179—181.
18. Von Kaulla KN, Schultz RL. Methods for evaluation of human fibrinolysis. *Am J Clin Pathol* 1958; 29: 104—112.
19. Gryglewski RJ. Interactions between endothelial mediators. *Pharmacol Toxicol* 1995; 77: 121—128.
20. Korbut R, Dąbroś W, Ocetkiewicz A, Gryglewski RJ. A biological method for studying the interaction between platelets and vascular endothelium. *Thromb Res* 1990; 57: 361—370.
21. Musiał J, Wilczyńska M, Sładek K, Cierniewski CS, Niżankowski R, Szczeklik A. Fibrinolytic activity of prostacyclin and iloprost in patients with peripheral arterial disease. *Prostaglandins* 1986; 31: 61—70.
22. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989; 86: 3375—3378.
23. Thiemermann C, Vane JR. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharide in the rat *in vivo*. *Eur J Pharmacol* 1990; 182: 591—595.
24. Madden KS, Sanders VM, Felte DL. Catecholamine influences and sympathetic neural modulation of immune responsiveness. *Ann Rev Pharmacol Toxicol* 1995; 35: 417—448.

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