The effect of the anabolic steroid stanozolol on tissue plasminogen activator activity and plasmin inhibition in the rat[1]

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Summary. The anabolic steroid stanozolol administered orally in male Sprague-Dawley rats induced a dose- and time-dependent increase in tissue plasminogen activator activity. The increase was highest in lung, lowest in heart, and intermediate in aorta and kidney. A slight increase in plasmin inhibition was noted only in the heart.

Drugs capable of inducing and sustaining a prolonged enhancement of fibrinolysis, and suitable for oral administration, might be of clinical importance in conditions with impaired fibrinolytic activity[2]. The anabolic steroid stanozolol is able to induce a long-lasting increase in blood fibrinolytic activity in man[3-6]. However, information about the effect of stanozolol on tissue fibrinolysis is lacking. Therefore, the present study was undertaken to investigate the effect of this anabolic steroid on the fibrinolytic activity of key organs in the rat.

Materials and methods. The anabolic steroid stanozolol (Stromba®, Winthrop, England) was administered daily p.o. in 36 adult, male Sprague-Dawley rats at the following doses: 0.1 mg/100 g/day (in 18 rats), and 0.2 mg/100 g/day (in 18 rats). In 36 control rats the inert carrier was administered p.o. The animals were sacrificed in groups 30, 60 and 90 days after the beginning of administration. In addition, 6 control rats were sacrificed before administration. Specimens of aorta, lung, heart and kidney were taken immediately after the animals were sacrificed. The specimens were briefly washed in saline, frozen and stored at $-70\,^{\circ}C$. Tissue sections from the same anatomical area of each organ of treated and control rats were studied histochemically with the fibrin slide technique for detection of plasminogen activator activity (PAA) and with the fibrin slide 'sandwich' technique for detection of plasmin inhibition, as both described before[7,8]. For this purpose, plasminogen-rich and plasminogen-free fibrinogen (Poviet, Organon-Teknika, The Netherlands), thrombin (Leo Pharmaceuticals, Denmark), and plasmin (Novo, Denmark) were used.

Results. Compared to controls, the average increase in the body weight was 3%, 6%, and 10% the 1st, 2nd and 3rd month, respectively, at the lower dose of stanozolol (in 15 out of 18 rats), while at the higher dose the increase was 10%, 15%, and 20% at the corresponding periods of time (in 16 out of 18 rats). In 5 out of 36 rats no increase in the body weight was noted until the end of the experiment, compared to controls.

In the control rats, the tissue with the highest PAA was the lung, followed by the medulla of the kidney, while the intima of the aorta showed the lowest PAA among the tissues studied. In myocardium and kidney cortex, the PAA was intermediate.

Among 18 rats treated with the lower dose of stanozolol (0.1 mg), 15 rats showed an anabolic response (body weight increase) and 12 of them responded with an increased PAA in lung and aorta, but not in heart and kidney. Among 18 rats treated with the higher dose (0.2 mg), 16 rats responded anabolically and 14 of them also fibrinolytically in all organs studied. The table shows the percentage of increase in tissue PAA, compared to controls, in fibrinolytic responders (a), and the percentage of increase in PAA by also taking into account the number of rats which responded anabolically but not fibrinolytically (b). In both cases the increase is statistically significant. A slight increase in plasmin inhibition was noted only in myocardium after 3 months' administration of stanozolol at the higher dose (in 14 rats).

Discussion. Among the treated rats, 86% responded anabolically to stanozolol and 84% of them (72% of the treated rats) showed a dose- and time-dependent increase in tissue PAA. Since stanozolol was administered p.o. individually, the small percentage of the non-responders might be due to a defective absorption or abnormal metabolism of the drug. The anabolic and/or fibrinolytic response might also depend upon such factors as the endocrine status of each animal. Human 'poor fibrinolytic responders' to anabolic steroids have been reported[9-11].

The fibrinolytic response to stanozolol was also dependent on the tissue. The highest and most consistent increase in PAA was noted in the lung and the lowest in the heart. In kidney and aorta, it was intermediate. The intima of the aorta in Sprague-Dawley rats exhibits a very low PAA. The enhanced intimal fibrinolytic activity shows the existence of a marked potential for increased activity. The tissue variation in the fibrinolytic response might be due to different distribution of specific steroid receptors in different tissues or to regional differences of the endothelium in the synthesis and/or release of plasminogen activator(s), as indicated by previous studies[12].

The increased vascular PAA found in key organs of the rat after oral administration of stanozolol correlates with the repeatedly reported increase of plasma fibrinolytic activity in humans after treatment with stanozolol alone[3-6] or in combination with phenformin[13-17] or metformin[18]. Therefore, increased vascular PAA could, at least partially, account for the increased blood fibrinolytic activity after administration of stanozolol as shown, for example, for the anabolic steroid ethylestrenol[9,10,19].

Stanozolol, at the doses and time used, did not affect tissue

Dose	0.1 mg/100 g/day						0.2 mg/100 g/day					
Time	30 days		60 days		90 days		30 days		60 days		90 days	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Lung	10%*	8%*	25%	19%	40%	30%	30%	26%	50%	44%	80%	70%
Aorta (intima)	–	–	–	–	40%	30%	30%	26%	40%	35%	60%	53%
Kidney cortex	–	–	–	–	–	–	20%	18%	50%	44%	60%	53%
Kidney medulla	–	–	–	–	–	–	10%*	9%*	30%	26%	40%	35%
Heart (myocardium)	–	–	–	–	–	–	–	–	10%*	9%*	20%	18%

* $p<0.01$, the rest $p<0.001$.

plasmin inhibition, except for a slight increase in the heart after 3 months administration at the higher dose. The reported results show that an anabolic steroid can affect vascular plasminogen activators without apparent effect on plasmin inhibitors.

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Evidence that endogenous vasopressin plays a protective role in circulatory shock. Role for reticuloendothelial system using Brattleboro rats

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Summary. Experiments performed on male Wistar, Long Evans and Brattleboro rats indicate that the latter strain of animals, lacking vasopressin in their posterior pituitaries, are extremely sensitive to hemorrhagic and bowel ischemic shock. Mild forms of both hemorrhagic and bowel ischemic shock, as produced in Wistar or Long Evans rats, results in marked hypotension, hemoconcentration and blockade of the reticuloendothelial system (RES) in Brattleboro animals of similar sex, age and weight. These direct findings indicate that release of endogenous vasopressin in shock syndromes may be critical in maintenance of circulatory homeostasis and RES function.

Several lines of investigation have revealed that hemorrhage, fluid loss and trauma are strong stimuli for the release of vasopressin from the pituitary glands of rats, cats and dogs[2]. This naturally occurring pituitary hormone is a potent constrictor of vascular smooth muscle cells[3], including arterioles and precapillary sphincters in the microcirculation[3,4]. It is not, however, known whether the release of vasopressin in circulatory shock is necessary for survival, since all of the previous experiments were done by indirect approaches (e.g., infusions of exogenous vasopressin)[5].

During the past decade, evidence has been gathered which suggests that tolerance to various types of circulatory shock, trauma and surgery is associated with the functional capacity of the phagocytic cells of the reticuloendothelial system (RES)[6]. Substances which depress the phagocytic powers of RE cells increase mortality, while materials which stimulate RE cell phagocytic activity is, in most instances, associated with increased tolerance to many forms of circulatory shock, trauma and systemic stress. Infusion of some vasopressin molecules into animals subjected to circulatory shock can enhance RES phagocytic activity and induce permanent survival[7].

In view of the importance of RE cells in host defense, and the question as to whether a lack of circulating vasopressin in shock alters RES function, the present experiments were undertaken. We now report here that Brattleboro rats with hereditary hypothalamic diabetes insipidus (lacking pituitary vasopressin) are exquisitely sensitive to the lethal effects of 2 different forms of circulatory shock when compared to rats of the parent strain (i.e., Long Evans) or normal age-matched Wistar rats. In addition, our findings indicate that circulatory shock induces a complete failure of RES phagocytic function in Brattleboro but not in normal (vasopressin-containing) rats.

Methods. 3 different groups of young, adult male rats (Brattleboro, Long Evans, Wistar strains, 230±40 g) were utilized. Prior to induction of circulatory shock, each Brattleboro rat was administered 3 ml of water, 100 g b.wt

Table 1. Exacerbation of mortality after shock in Brattleboro rats

Rat strain	Hemorrhage Survivors/ total	% Survival	Bowel ischemia Survivors/ total	% Survival
Wistar	16/24	75	16/20	80
Long Evans	12/18	67	14/20	70
Brattleboro	1/12	8*	1/10	10*

* Significantly different from Wistar and Long Evans ($p < 0.01$).