Release of Plasminogen Activator from Rat Liver Lysosomes Induced by Stress Related Enzymes* (33896)

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Acid phosphatase and beta glucuronidase are among the hydrolytic enzymes contained in the lysosomes of mammalian cells (1). Lysosomal enzymes appear to be released and activated in proportion to the permeability of the membrane enclosing them (2, 3). *In vitro* manipulations such as subjection to nonionic detergents, vitamin A, or repeated freezing and thawing (4, 5) will increase the pemeability of these organelles and release their enzymes.

Stress produces a similar effect *in vivo*. Janoff and co-workers (3) reported increased release of acid phosphatase and beta glucuronidase from lysosomes isolated from the liver of rats subjected to endotoxin shock and drum trauma. Drum trauma also initiates an increase in blood proteolytic activity as does stress generally (6). This rise is preceded by the development of hypercoagulability in the blood of the shocked animal (7). In this study rats were injected with these enzymes to mimic the body's response to stress and to find what effect such injections would have on the release of lysosomal enzymes into the blood.

Lysosomes have been reported to store an enzyme which converts the blood protein, plasminogen, into the fibrinolytic enzyme plasmin (8–11). Increased release of plasminogen activator from the lysosomes following stress may be essential to combating the heightened blood coagulability which follows the onset of shock (12).

Serotonin is an amine which is found in peripheral blood in significant amounts associated with the platelets. In blood clotting the disintegrating platelets release serotonin causing general vasoconstriction (13). Its release from platelets presumably is heightened in stress coincident with the rise in blood coagulability. Serotonin injections have been used by Kwaan and co-workers (13) to initiate an increase in blood fibrinolytic activity causing rapid digestion of experimentally produced clots in the veins of rabbits. Finally, in this study, we injected rats with serotonin to assess its effect on the release of lysosomal enzymes into the blood. Blood plasma and liver lysosomes of rats injected with acid phosphatase, beta glucuronidase, serotonin, or physiological saline were analyzed for plasminogen activator content. Acid phosphatase levels in these tissues were measured also for additional evidence of changes in lysosomal permeability.

Methods. I. Acid phosphatase study. Albino, male rats (Sprague-Dawley strain) weighing from 425–460 g were divided into two groups of 10. The first group (control group) was injected intraperitoneally with 1.0 ml of physiological saline. Each member of the second group was injected with 10 mg of acid phosphatase dissolved in 1 ml of physiological saline. The acid phosphatase was derived from wheat germ (Nutritional Biochemical Corporation). One hr after the injections, the rats were anesthetized with 6% Nembutal injections given intraperitoneally. Blood samples were drawn from the abdominal aorta. Liver segments (1 g) were homogenized with a Potter-Elvehjem apparatus in 3 ml of 0.25 *M* sucrose and lysosomes separated by the differential centrifugation method of De Duve *et al.* (1).

Lysosomal fractions and blood sera from control and enzyme injected rats were analyzed for acid phosphatase content by Shinowara's method (14), a modification of the Bodansky procedure (15).

Fibrinolytic activity and plasminogen activator activity of these tissues were assayed using fibrin clots tagged with ^{131}iodine. The

* Supported by the Louisiana Heart Association and the Cancer Association Research of Greater New Orleans.

clots were made according to the procedure of Alkjaersig *et al.* (16).

Each clot contained 0.4 ml of human blood plasma, 0.1 ml of fibrinogen ^{131}I, and 0.1 ml of thrombin (5 units, Parke Davis Co.) To determine fibrinolytic activity, fibrin clots tagged with ^{131}I and made with aged human plasma (approx 1 year old) were used. Contamination of the clots with plasminogen was thereby minimized. One tenth ml of the tissue (plasma or lysosome suspension) was added to the clot. The mixture was incubated for 30 min in a shaking water bath at 37°. The radioactivity of a 0.1-ml aliquot of the clot supernate was calculated as a percentage of the radioactivity of the whole clot. Controls in which 0.1 ml of physiological saline replaced tissue fractions were included to assess spontaneous lysis of the clots. Determinations of plasminogen activator were similar to those for fibrinolytic activity except that bovine plasminogen (0.1 mg/clot in 0.1 ml of saline) from the Nutritional Biochemical Corporation was added to each clot. Saline controls were included as above using plasminogen-enriched clots with saline added in place of tissue. Results for fibrinolytic activity and plasminogen activator activity are reported as percentage digestion of the plain and plasminogen-enriched clots, respectively. A plasminogen activator control was also included consisting of 100 units of Streptokinase (Lederle-Varidase) added to the plasminogen enriched clots (17).

II. Serotonin study. Two groups of 10 rats similar in every way to those used in the acid phosphatase study were used. Each member of one group received an intraperitoneal injection of 0.016 mg of serotonin creatinine sulfate (Nutritional Biochemical Corporation, 5 hydroxytryptamine creatinine sulfate, monohydrate) dissolved in 1 ml of physiological saline. The second (control) group was injected ip with 1 ml of physiological saline. After 1 hr all were anesthetized and their tissues were analyzed in identical manner to that of the acid phosphatase study.

III. Beta glucuronidase study. Rats of the type described were divided into one group of 14 which received an ip injection of 400 units of beta glucuronidase (Nutritional Biochemical Corporation, 25,000 Fishman units/g) dissolved in 1 ml of physiological saline each day for 7 consecutive days. The second (control) group was so injected with 1 ml of physiological saline. Blood serum and liver lysosomes were analyzed as before: 1 hr past final injection for plasminogen activator activity. Acid phosphatase levels in those tissues were analyzed in this group by the method of Babson *et al.* (18). Results of this assay are reported in Table III as international units of enzyme per milliter. This unit is equivalent to approximately 0.18 Bodansky units of acid phosphatase activity reported in Tables I and II.

Results. Lysosomes from the liver of acid phosphatase-injected rats possessed significantly smaller levels of plasminogen activator and acid phosphatase than those of saline-injected controls (Table I). One hr postinjections, acid phosphatase activity in liver lysosomes fell from 1.3 mg of P/100 ml of tissue in controls to 0.7 in enzyme-treated animals. Simultaneously, blood levels rose from 0.2 to 0.5 mg of P/100 ml of tissue. Plasminogen activator content of liver lysosomes of control rats lysed 26.4% of plasminogen enriched clots whereas only 22.8% was digested by those of enzyme injected rats. In the blood, plasminogen activator coincidently rose from that amount yielding 25.3% lysis to a level causing 35.5% clot dissolution. Spontaneous digestion of these clots accounted for 14.2%. Streptokinase (100 units) included the activation of the plasminogen within these clots and resulted in 75.8% digestion under the same test conditions. Enzyme treatment did not appear to increase the fibrinolytic activity of the blood serum. Increased plasmin levels which developed in the blood of the enzyme-induced animals in response to the rise in blood plasminogen activator content was not measurable in the fibrinolytic activity assay presumably because of the abundance of plasmin inhibitors in the circulating blood of higher animals (19). These may prevent a rise in blood proteolytic activity without impeding activation of fibrin bound plasminogen and subsequent clot digestion.

Lysosomal enzymes are not known to be

TABLE I. Effects of Acid Phosphatase Injection on Rat Tissues.[a]

	Fibrinolytic activity[b]		Plasminogen activator activity[c]		Acid phosphatase activity[d]	
	Serum	Lysosomes	Serum	Lysosomes	Serum	Lysosomes
Control rats	18.0 ± 0.1	18.3 ± 0.3	25.3 ± 0.2	26.4 ± 0.3	0.2 ± 0.02	1.3 ± 0.03
Acid phosphatase injected rats	18.4 ± 0.3	18.4 ± 0.4	35.5 ± 0.2	22.8 ± 0.3	0.5 ± 0.02	0.7 ± 0.03
p value[e]			<0.001	<0.001	<0.001	<0.001

[a] Mean values for 10 rats/datum.

[b] Percentage lysis of Fibrin ^{131}I clot; spontaneous lysis of these clots in the absence of added tissue yielded 16.2% digestion.

[c] Percentage lysis of Fibrin ^{131}I clots enriched with plasminogen; spontaneous digestion of these clots in the absence of tissues produced 14.5% digestion; 100 units of streptokinase produced 75.8% lysis after 30 min at 37°.

[d] Activity reported as mg of phosphorus released per 100 cc of the tissue sample (Bodansky units).

[e] Probability determined by the Student's *t* test denotes significance of the difference between values for control and acid phosphatase injected rats.

fibrinolytic in themselves. Therefore, a rise in fibrinolytic activity of the lysosomes of enzyme-injected animals would not be expected. Fibrinolytic values for sera and lysosomes of test and control groups yielded about 18% digestion of plasminogen-poor, fibrin ^{131}I clots, just above the value for the spontaneous lysis control, 16.2%. Injections of serotonin (0.016 mg/cc/rat) affected the lysosomes of rat liver (Table II) in a manner very similar to acid phosphatase input (10 mg/cc/rat). Acid phosphatase content of the lysosomes fell from a mean assay measurement of 1.3 mg of P/100 cc of tissue in saline-injected controls to 0.9 in serotonin-injected rats. The blood levels rose, respectively, from a value of 0.2 to 0.7. Plasminogen activator activity decreased from 25.8% lysis by control lysosomes to 23.6% in those from enzyme-treated animals and rose in the blood from 25.5% to 27.8% in the two groups. Fibrinolytic activity again was close to the value for spontaneous lysis of plasminogen-poor clots with saline added in place of tissue

TABLE II. Effects of Serotonin Injections on Rat Tissues.[a]

	Fibrinolytic activity[b]		Plasminogen activator activity[c]		Acid phosphatase activity[d]	
	Serum	Lysosomes	Serum	Lysosomes	Serum	Lysosomes
Control rats	18.5 ± 0.2	17.5 ± 0.3	25.5 ± 0.3	25.8 ± 0.4	0.2 ± 0.02	1.3 ± 0.03
Serotonin injected rats	18.7 ± 0.3	17.0 ± 0.4	27.8 ± 0.4	23.6 ± 0.3	0.7 ± 0.03	0.9 ± 0.03
p value[e]	—	—	<0.003	<0.003	<0.001	<0.001

[a] Mean values for 10 rats/datum.

[b] Percentage lysis of Fibrin ^{131}I clots; spontaneous lysis of these clots with saline added in place of tissues gave rise to 16.1% digestion.

[c] Percentage lysis of Fibrin ^{131}I clots enriched with plasminogen; spontaneous lysis controls produced 14% digestion; 100 units of streptokinase induced 75.5% lysis.

[d] Activity reported as mg of phosphorus released by 100 cc of tissue (Bodansky units).

[e] Probability determined by the Student's *t* test; comparison of control and enzyme injected values in the respective columns.

TABLE III. Effects of Beta Glucuronidase Injections in Rat Tissues.[a]

	Fibrinolytic activity[b]		Plasminogen activator activity[c]		Acid phosphatase activity[d]		Protein content (g/100 ml)[e]	
	Serum	Lysosomes	Serum	Lysosomes	Serum	Lysosomes	Serum	Lysosomes
Control rats	18.2	18.3	24.3 ± 1.2	26.8 ± 1.5	2.1 ± 0.1	8.5 ± 0.2	6.5	0.9
Beta glucuronidase injected rats	18.5	18.0	28.5 ± 1.5	18.6 ± 1.8	2.6 ± 0.1	13.5 ± 0.3	6.4	1.5
p	—	—	<0.003	<0.001	—	<0.003		

[a] Mean values for 14 rats/datum.

[b] Percentage lysis of Fibrin ^{131}I clots.

[c] Percentage lysis of Fibrin ^{131}I clots enriched with plasminogen.

[d] Activity reported as international units of enzyme per milliliter which will liberate 1 μmole of alpha naphthol/min.

[e] Biuret determination of protein content of tissues.

(17–18.7% for the tissue vs. 16.1% control digestion).

Beta glucuronidase (400 units/ml/rat) injected once daily for 7 days also caused the plasminogen activator content of the liver lysosomes to fall (Table III). Those of control rats digested 26.8% of the plasminogen enriched clots and those from enzyme-injected rats only digested 18.6%.

The activator activity in blood serum rose from that yielding 24.3% digestion to 28.5% in enzyme-treated rats. Acid phosphatase increased from 2.1 units (18) in the controls to 2.6 units in the beta glucuronidase-injected animals. On the other hand in contrast to the results in the acute studies with acid phosphatase and serotonin injections, acid phosphatase content of the lysosomes was greater (13.5 units) following beta glucuronidase treatment than in those from control rats (8.5 units). The protein content of the lysosomes was also greater in the enzyme treated rats (1.5 g/100 ml) than in the controls (0.9 g/100 ml).

It appears from this work that acid phosphatase, serotonin, and beta glucuronidase may be used as experimental tools to increase lysosomal permeability and in so doing to increase blood levels of plasminogen activator. There is also the possibility that altered clearance of plasminogen activator has contributed to these findings. Plasminogen activator is reported to be cleared from blood plasma by the liver (20). The clearance rate is said to vary following electroshock or nicotinic acid injection in normal humans and more pronouncedly in hepatic cirrhosis patents. A fall in liver lysosome content of plasminogen activator with a coincident rise in its blood level could conceivably reflect diminished liver clearance of the enzyme. The ability to control plasminogen activator levels in the blood by use of acid phosphatase, serotonin, or beta glucuronidase may be important in the alleviation of thromboembolic disorders since, β-glucuronidase injections already have been used successfully to depress lipemia and blood hypercoagulability in atherosclerotic patients (21).

Summary. Different groups of rats were injected with acid phosphatase, beta glucuronidase, and serotonin in the study. An hour after injection the levels of plasminogen activator and acid phosphatase activity were significantly increased above those of saline-injected control rats with the exception of acid phosphatase activity in sera of beta glucuronidase-injected rats. Simultaneously, the lysosomal fractions isolated from the liver of these rats showed a significant decrease in plasminogen activator and acid phosphatase activity as compared with those of saline-injected controls. These enzymes may be helpful as experimental inducers of plasmino-

gen activator release from cellular lysosomes into the blood vascular system in higher animals.

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Received Nov. 13, 1968. P.S.E.B.M., 1969, Vol. 131.