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Hepatic Necrosis Induced by Norepinephrine in Rabbits[1] (42570)

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Abstract. Extensive hepatic necrosis was produced in rabbits 48 hr following infusion of a cardiopathogenic dose of norepinephrine (NE, 2 μg/kg/min for 90 min). Livers had necrotic areas of varying sizes and gross appearances. Histologically, the lesions were areas of lytic–coagulative necrosis with massive mineralization by calcium. In addition, the serum glutamate–pyruvate transaminase (GPT) was significantly elevated ($P < 0.001$). Pretreatment with the α_1-adrenoceptor blocker prazosin (200 μg/kg) 15 min prior to the standard NE infusion prevented both liver necrosis and serum GPT elevation. It is concluded that large doses of NE produce tissue injury in the liver. This may be the result of excessive activation of the α_1-adrenoceptor system, which leads to hepatic ischemia and necrosis.

It has been known for many years that administration of large doses of catecholamines causes marked destructive changes in cardiac muscle (1, 2). The histologic lesions produced by these sympathomimetic agents includes myofibrillar degeneration, focal myofiber necrosis, and leukocytic infiltration (3, 4). A number of possible mechanisms have been proposed to explain the pathogenesis of catecholamine-induced myocardial injury and necrosis (5–7). However, there is little information in the literature concerning the pathogenic effects of catecholamines on other organs.

Recent studies from our laboratory have shown that norepinephrine-induced cardiac lesions in rabbits are largely the result of activation of the α_1-adrenoceptor system (8). Pharmacological evidence has indicated that the liver contains both α- and β-adrenoceptors, specifically α_1-adrenoceptors, which are stimulated by both epinephrine and norepinephrine (9, 10). Activation of sympathetic nerves to the liver (11) or intravenous infusion of epinephrine and norepinephrine (12) causes a significant increase in hepatic arterial vascular resistance. In view of these observations, studies were undertaken to investigate whether infusion of a cardiopathogenic dose of norepinephrine would also cause hepatic lesions in rabbits and to assess whether pretreatment of animals with an α_1-adrenoceptor blocker would ameliorate the NE-induced hepatic necrosis as has been observed in the heart (8).

Materials and Methods. A total of 20 adult New Zealand white rabbits were used for this study. All animals were anesthetized with sodium pentobarbital, 30 mg/kg by ear vein injection. Femoral artery and vein were cannulated with polyethylene catheters for the monitoring of arterial blood pressure and heart rate and for the infusion of the drug, respectively. Body temperature was maintained with a heating pad. Arterial blood samples were obtained at 30-min intervals for analysis of pH, blood gases (Radiometer Blood Gas Analyzer, Copenhagen), serum electrolytes (Photovolt Electrolyte Analyzer System PV A-4, Dow Chemical), and serum glutamate–pyruvate transaminase (GPT, Sigma).

Norepinephrine (NE Levophed, Winthrop) was freshly prepared by dilution with normal saline to a concentration which would provide a dose of 2 μg/kg/min at a constant infusion rate of 0.382 ml/min for 90 min. It has been demonstrated in earlier studies that this dose produces a consistent pattern of cardiac lesions and impairment of ventricular function in rabbits 48 hr after infusion (13, 14). The α_1-adrenoceptor blocker prazosin (200 μg/kg), when employed, was given 15 min prior to the beginning of norepinephrine infusion. This dose effectively reduces NE-induced (2 μg/kg/min for 90 min) cardiac lesions (8).

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After completion of infusion, the catheters were removed, the femoral wounds were surgically closed, and the animals were returned to their cages for recovery. Forty-eight hours later the rabbits were anesthetized again, and blood samples were obtained. The rabbits were then killed with an overdose of pentobarbital. The livers were immediately removed, grossly examined, weighed, and tissue samples were taken and fixed in 10% buffered formalin for histopathology. These were prepared by standard histologic methods and stained with hematoxylin and eosin (H & E) and Von Kossa for subsequent microscopic analysis.

Nine rabbits were infused with NE (2 μg/kg/min for 90 min) and killed after 48 hr. Five rabbits were given the α_1-adrenoceptor blocker prazosin (200 μg/kg) prior to the standard NE infusion and were also killed after 48 hr. Three rabbits were given normal saline in lieu of NE and killed at 48 hr. Three normal rabbits were killed and used as gross and histologic references.

The gross necrotic areas of the liver were examined and graded using a scoring system described in detail elsewhere (15). In brief, a score of 0 was given when no gross abnormality was present; those with a few foci of necrosis were given a score of 1.0. Those judged to have more numerous foci of necrosis than those graded 1.0 or lesions of greater size in one lobe were given a score of 2.0. Those with flecked or spotty widely scattered necrosis involving more than one lobe were given a score of 3.0. Those with more intense or massive necrosis than those graded 3.0 were given a score of 4.0. A score of 5.0 was given to those livers with massive necrosis as extensive as those graded 4.0, but with at least one whole lobe completely involved.

All data were analyzed by standard statistical methods; one-way analysis of variance was performed for multiple group comparisons, followed by Duncan's new multiple range test, to identify significant differences among the various pairs (16). For each test statistical significance was determined at the 5% level of probability.

Results. Figure 1 compares the gross appearance of livers from a saline-infused control and a NE-infused rabbit killed 48 hr following the infusion. The control liver has normal contours and a uniform reddish-brown color (Figure 1A). None of the control animals had any grossly identifiable liver necrosis and all were graded 0. In contrast, 6 of 9 rabbits receiving the standard dose of NE (2 μg/kg/min for 90 min) had markedly abnormal livers. The livers were pale, light brown, and had irregularly shaped areas of necrosis. These were on both the anterior and posterior surfaces, usually involved all lobes (Figure 1B and 1C), and were more severe toward the periphery. Necrotic areas often become confluent forming large necrotic areas.

The mean liver gross necrotic scores from all groups are summarized in Fig. 2. The mean necrotic score was 2.9 in the standard NE-infused animals. This may be compared with the mean score of 0.70 in rabbits pretreated with pretreatment of prazosin (200 μg/kg). No evidence of liver engorgement was noted in NE-infused rabbits as compared to other groups. The average wet liver weight and the ratio of liver wet weight to body weight were almost identical in all groups (Table I).

The severity of histologic changes in NE-infused livers varied with the sampling distance from the gross necrotic site. In general, necrosis, when present, was always maximal in zone 3 and expanded into zone 2 with increasing damage (Fig. 3). Mineralization was commonly seen in areas of most intense necrosis. The Van Kossa stain revealed the mineral deposits in necrotic areas to be composed largely of calcium. In contrast, histologic examination of livers from prazosin-pretreated rabbits showed no zonal necrosis, but only slight congestion, moderate lipid degeneration, and a few isolated dead cells in zone 3 (Fig. 4). No control animals had any histologic changes in the liver.

The extent of NE-induced liver necrosis was also assessed by measuring the levels of serum GPT. As shown in Fig. 5, 2 days following standard NE infusion the average levels of serum GPT in rabbits increased significantly to 200 IU/liter from the control values of 19 IU/liter. However, when rabbits were given prazosin 15 min prior to the standard NE infusion the average serum GPT levels were only slightly elevated above the control values, and this change was not statistically significant. It should be noted that the serum GPT activity was not altered 10 min postinfusion in all groups. Furthermore, no measurable changes of serum GPT were found in control rabbits at 10 min or 48 hr after saline infusion.

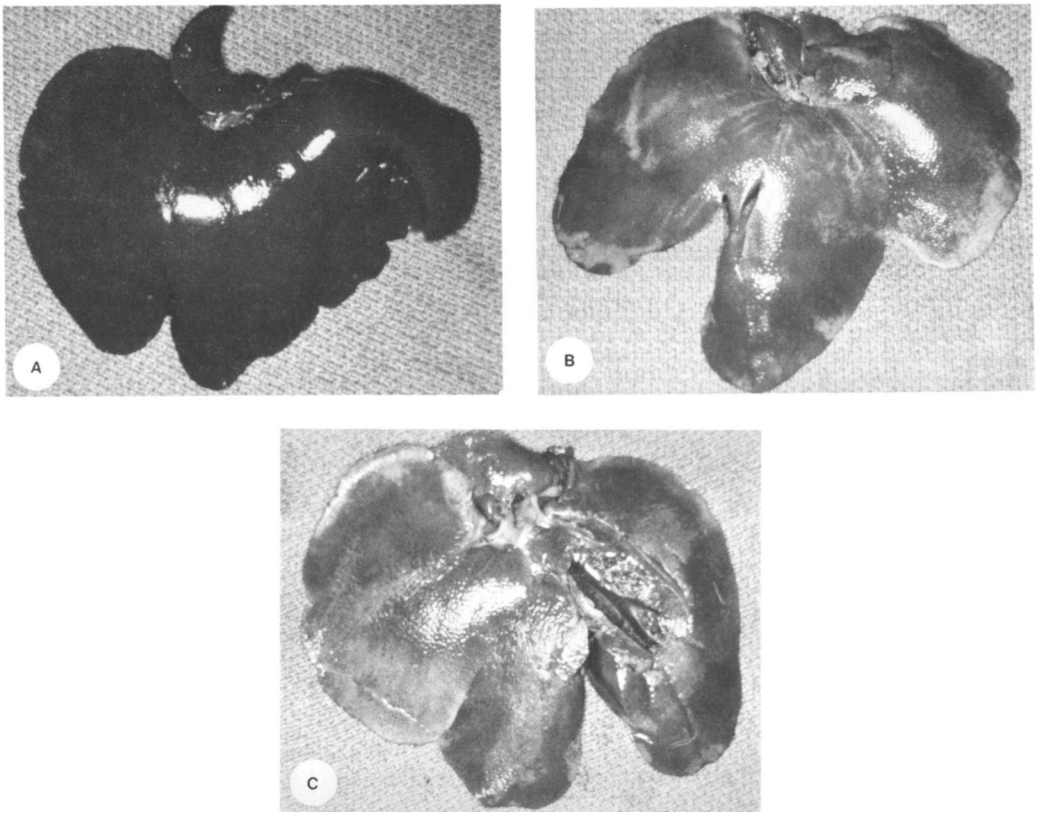

FIG. 1. Gross appearance of a saline-infused control rabbit liver (A) and the anterior (B) and posterior (C) aspects of a liver from a NE-infused rabbit (2 μg/kg/min for 90 min). Note the pronounced necrotic injury in the NE-infused liver and characteristic lesions (score 4.0).

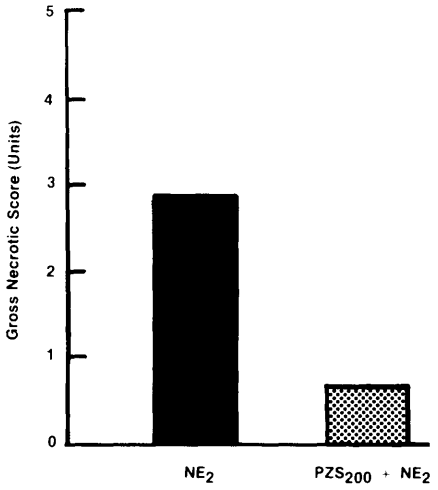

FIG. 2. Gross necrotic scores from livers in groups given norepinephrine only (NE_2, 2 μg/kg/min; $N = 9$) or prior adrenergic blockade with the α_1-adrenoceptor blocker, prazosin (PZS, 200 μg/kg; $N = 5$).

Discussion. It is well established that catecholamines given in high concentrations produce myocardial injury and necrosis. The morphologic features, severity, and extent of myocardial lesions vary substantially with the dose, species, and the time lapse following the drug administration (1, 4, 13). A reproducible

TABLE I. ANATOMIC DATA

Group	*N*	BW	Liver wt.	Liver wt. / BW
Saline	3	2.3 ± 0.2	72.8 ± 5.1	3.2 ± 0.1
NE_2	9	2.3 ± 0.1	78.1 ± 5.5	3.3 ± 0.2
$PZS_{200} + NE_2$	5	2.3 ± 0.1	72.0 ± 3.1	3.0 ± 0.2

Note. Values are means ± SE. N = Number of animals. BW = body weight (kg). Liver wt. = liver weight (g). NE_2 = infusion of norepinephrine at 2 μg/kg/min for 90 min. PZS_{200} = pretreatment of prazosin 200 μg/kg.

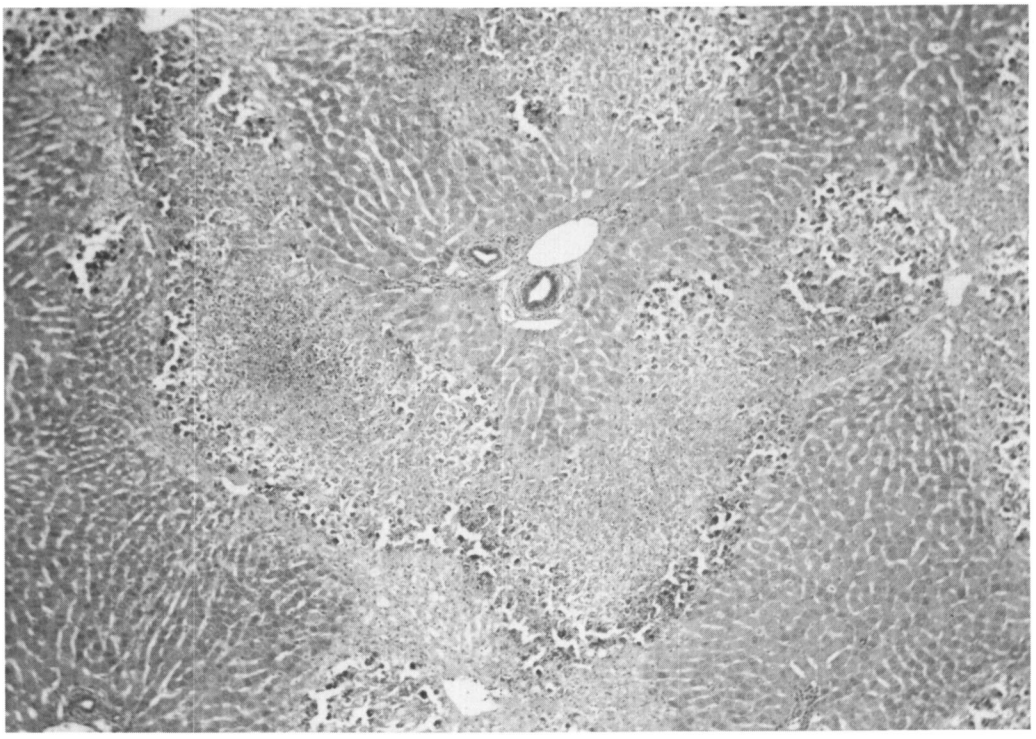

FIG. 3. Liver from a rabbit infused with standard NE (2 μg/kg/min for 90 min) and examined 2 days later. Extensive necrosis and mineralization are seen in zone 3 (H & E ×205).

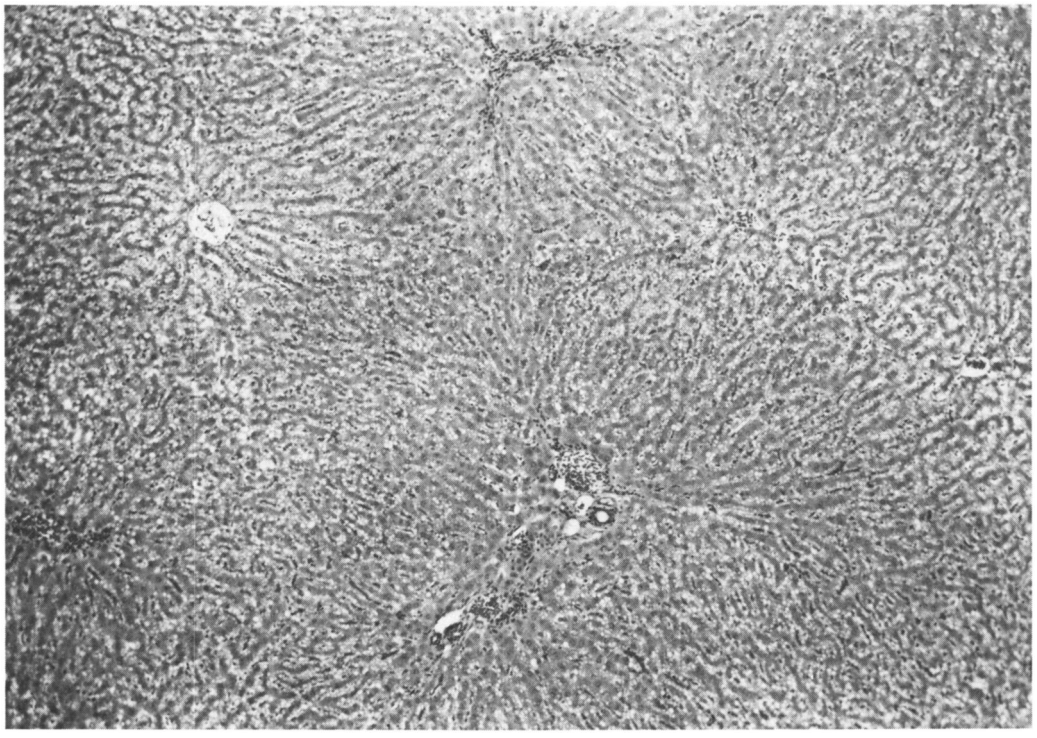

FIG. 4. Liver from a rabbit given 200 μg/kg prazosin prior to the standard NE infusion. In contrast to Fig. 3, note the absence of NE-induced hepatic necrosis (H & E ×205).

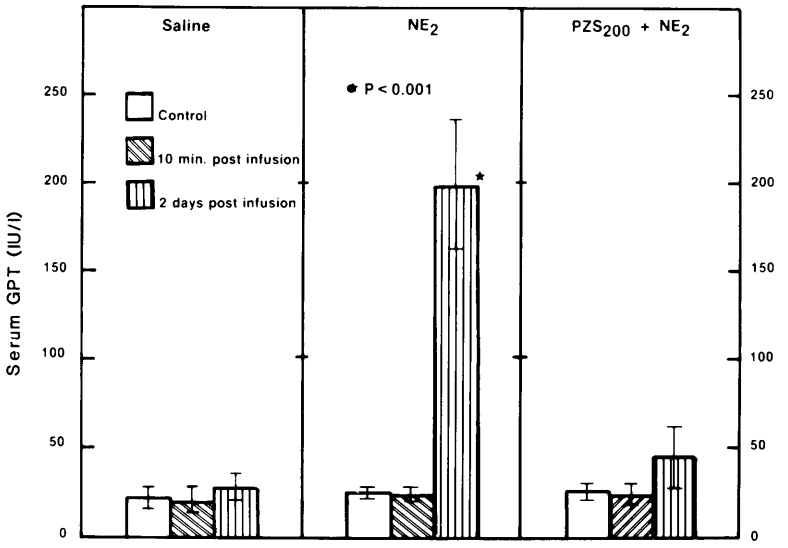

FIG. 5. Alteration of serum GPT levels with time in rabbits following saline infusion ($N = 3$), standard norepinephrine infusion (NE_2, 2 μg/kg/min for 90 min; $N = 9$), and standard NE_2 infusion with pretreatment of prazosin (PZS_{200}, 200 μg/kg; $N = 5$). Vertical brackets shows SE. Values significantly ($P < 0.001$) different from control values are indicated by the asterisk.

model of NE cardiomyopathy in rabbits has been developed in our laboratory using a short term (90 min) infusion of NE, given in a dose of 2 μg/kg/min. This produces a consistent pattern of cardiac injury readily identifiable by histologic examination and functional studies in the heart 48 hr after the infusion (8, 13, 14). We have also recently shown that β-adrenoceptor blockers do not prevent NE-induced cardiac damage in rabbits, but that α-adrenoceptor blockers effectively prevent the appearance of myocardial lesions (8, 17).

The results of the present study clearly show that the administration of a cardiopathogenic dose of NE (2 μg/kg/min for 90 min) in rabbits can also induce massive necrotic damage in the liver. Concurring with findings in the heart, the NE-induced hepatic lesion could also be prevented by the pretreatment of prazosin, based upon gross and histologic examination and measurement of serum GPT levels. The livers of rabbits killed 48 hr after NE infusion had extensive necrosis (Figs. 1, 2, and 3), and the serum levels of GPT were significantly elevated (Fig. 5). Pretreatment with prazosin prevented both liver necrosis and serum GPT elevation (Figs. 3, 4, and 5). The level of serum GPT has proven to be a specific and sensitive indicator of liver cell damage and is frequently quantitated in serum as an index of liver cell necrosis (18).

One hypothesis that might explain the mechanism of NE-induced necrosis in the liver involves hepatic vasoconstriction through massive activation of α-adrenergic pathways, sufficient to cause ischemic damage to the liver. Both α- and β-adrenoceptors are present in the hepatic vasculature. Using a rat microvasculature preparation, Reilly *et al.* (19) demonstrated that electrical stimulation of the nerves to the liver elicited α-mediated constriction of portal venules, sinusoids, and hepatic arterioles. Topical application of the α-blocker phentolamine abolished this response. However, following α blockade and electrical stimulation of hepatic nerves, there were no alterations in blood flow through the microvasculature. They suggested that the major influence of adrenergic regulation on hepatic blood flow is mediated via the α-adrenoceptor system. Infusion of epinephrine and norepinephrine also caused a profound increase in hepatic arterial vascular resistance (11, 12, 19).

At present the pathogenesis of NE-induced hepatic necrosis is unclear. Histologic evidence supports the concept that the hepatic necrosis

may be secondary to ischemia. As shown in Fig. 3, the NE-induced hepatic necrosis was always maximal in zone 3, which is the microcirculatory periphery of the liver acinus. Cells in this area are particularly vulnerable to ischemia and dietary deficiency. It is of interest to note that Chien *et al.* (20) found that rat livers subjected to 30 min of ischemic occlusion show little or no histologic evidence of liver cell death 24 hr after reflow. In contrast, livers subjected to a longer period of ischemic occlusion (2–3 hr) revealed extensive necrosis when examined 24 hr after reflow. Intracellular calcium accumulation has also been observed in irreversible liver injury produced by ischemia (21) and many hepatotoxins (22).

In this study, the Von Kossa stain showed extensive Ca^{2+} deposition in NE-infused rabbit liver, but not in saline-infused and NE-infused prazosin-pretreated rabbits. It should be noted that the accumulation of calcium in irreversible cellular damage occurred only when arterial blood flow was re-established, but not in tissues which were not reperfused with arterial blood (20, 23). The possible involvement of free radicals and membrane lipid peroxidation in liver cell injury caused by ischemia-reperfusion has also been demonstrated (24). In addition, catecholamines readily undergo oxidation, and it has been suggested that the adrenochrome or other oxidative metabolites of catecholamines may be directly responsible for catecholamine-induced cell injury (7). All these considerations will clearly require further investigation to establish the precise cellular mechanisms of NE-induced hepatic necrosis and to determine the significance of excessive activation of the endogenous adrenergic system and stress in potentiation of chemical and biological agents which induce hepatic injury (25).

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