Role of α_1-Adrenoceptors in Norepinephrine-Induced Cardiomyopathy

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This study practically delineated the contribution of α-adrenoceptor activation to the pathogenesis of norepinephrine (NE) cardiomyopathy. A total of 64 adult New Zealand white rabbits were used. NE cardiomyopathy was produced in rabbits by a 90-minute intravenous infusion of norepinephrine (2 μg/kg/min at infusion rate 0.382 ml/min). Arterial blood pressure and heart rate were constantly monitored. Arterial blood samples were obtained at 30-minute intervals for measurements of pH, blood gases, and glucose. Alpha-adrenoceptor blocking agents, when employed, were given 15 minutes prior to the initiation of NE infusion. Two days after treatment the rabbits were killed. The hearts were examined microscopically and assigned a histologic score. Pretreatment with the α_1-adrenoceptor blocker prazosin at 50, 100, or 200 μg/kg significantly reduced NE-induced myocardial injury in a dose-related manner. In contrast, the presence of α_2-adrenoceptor blocker yohimbine at 2.5 or 5.0 mg/kg was ineffective in preventing the formation of myocardial lesions. These findings suggest that NE cardiomyopathy may result largely from activation of the α_1-adrenoceptor system in the rabbit model. (Am J Pathol 1985, 121:316–321)

LARGE DOSES of catecholamines produce myocardial damage in experimental animals[1,2] as well as in humans.[3,4] The morphology of catecholamine-induced myocardial lesoins has been fully described in the literature,[1,3,5] and potential pathogenic mechanisms have been proposed.[6,7] The operating assumption of most studies has been that catecholamine injury involves β-adrenergic pathways.

It is a well-known fact that norepinephrine (NE) is both an alpha and beta agonist. Substantial evidence indicates the existence of α-adrenergic receptors in cardiac muscle in addition to beta receptors.[8–11] However, the potential contribution of α-adrenoceptor activation to the pathogenesis of catecholamine cardiomyopathy has not been fully elucidated.

Recently Downing and Lee[12] reported that a short-term (90-minute) infusion of NE (2 μg/kg/min) or methoxamine (15 μg/kg/min) in rabbits results in severe myocardial damage and leukocytic infiltration in the heart 48 hours after infusion. Beta blockade with practolol (4 mg/kg) and propranolol (1 mg/kg) failed to significantly reduce cardiac injury after norepinephrine. However, nonselective alpha blockade with phentolamine (10 mg) markedly reduced the severity of the lesions. The purpose of this study was to examine the contribution of subtype α-adrenoceptor activation to the pathogenesis of NE-induced myocardial injury in rabbits with the highly preferential α_1, α_2-antagonists prazosin and yohimbine, respectively.

Materials and Methods

Sixty-four adult New Zealand white rabbits were used for this study. All animals were anesthetized with sodium pentobarbital, 30 mg/kg, by ear vein injection. The femoral artery was cannulated with polyethylene catheters for the measurement of arterial blood pressure and heart rate with the use of a Sanborn transducer and Grass Polygraph system (Model 7), and the femoral vein was cannulated for the infusion of drug. Body temperature was maintained with a heating pad. Arterial blood samples were obtained at 30-minute intervals for analysis of pH and blood gases (Radiome-

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ter Blood Gas Analyzer, Copenhagen). Arterial hematocrit and blood glucose also were determined.

Norepinephrine (Levophed, Winthrop Laboratories, New York, NY) was freshly prepared by dilution in normal saline to concentrations which would provide a dose of 2 μg/kg/min at a constant infusion rate of 0.382 ml/min for 90 minutes. It has been demonstrated in earlier studies[12-14] that this dose produces a consistent pattern of cardiac lesions in the rabbit model. Alpha-adrenergic blocking agents, when employed, were given 15 minutes prior to the beginning of NE infusion. These included prazosin (50, 100, or 200 μg/kg) and yohimbine (2.5 or 5.0 mg/kg).

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-one were killed 48 hours later with an overdose of pentobarbital; 6 were killed 6 hours and 8 were killed 2 hours after completion of NE infusion. Nine rabbits were given normal saline instead of NE and were killed at 48 hours as untreated controls. The hearts were immediately removed, and a middle transverse section of the left ventricle (free wall plus septum) was obtained and fixed in 10% buffered formalin. Hearts were prepared by standing histologic methods and stained with hematoxylin and eosin (H&E) for subsequent microscopic examination.

Histologic grading was done as previously described.[13] In brief, a score of 2.0 (Figure 1) was given to those ventricles with florid and widespread cellular infiltration; those with definite but sparse lesions were scored 1.0. Intermediate lesions were scored 1.5. Sections with lesions less extensive than those graded 1.0 were given score of of 0.5. A score of 0 was given when no histologic abnormality was present.

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 of variables.[15] For each test statistical significance was determined at the 5% level of probability.

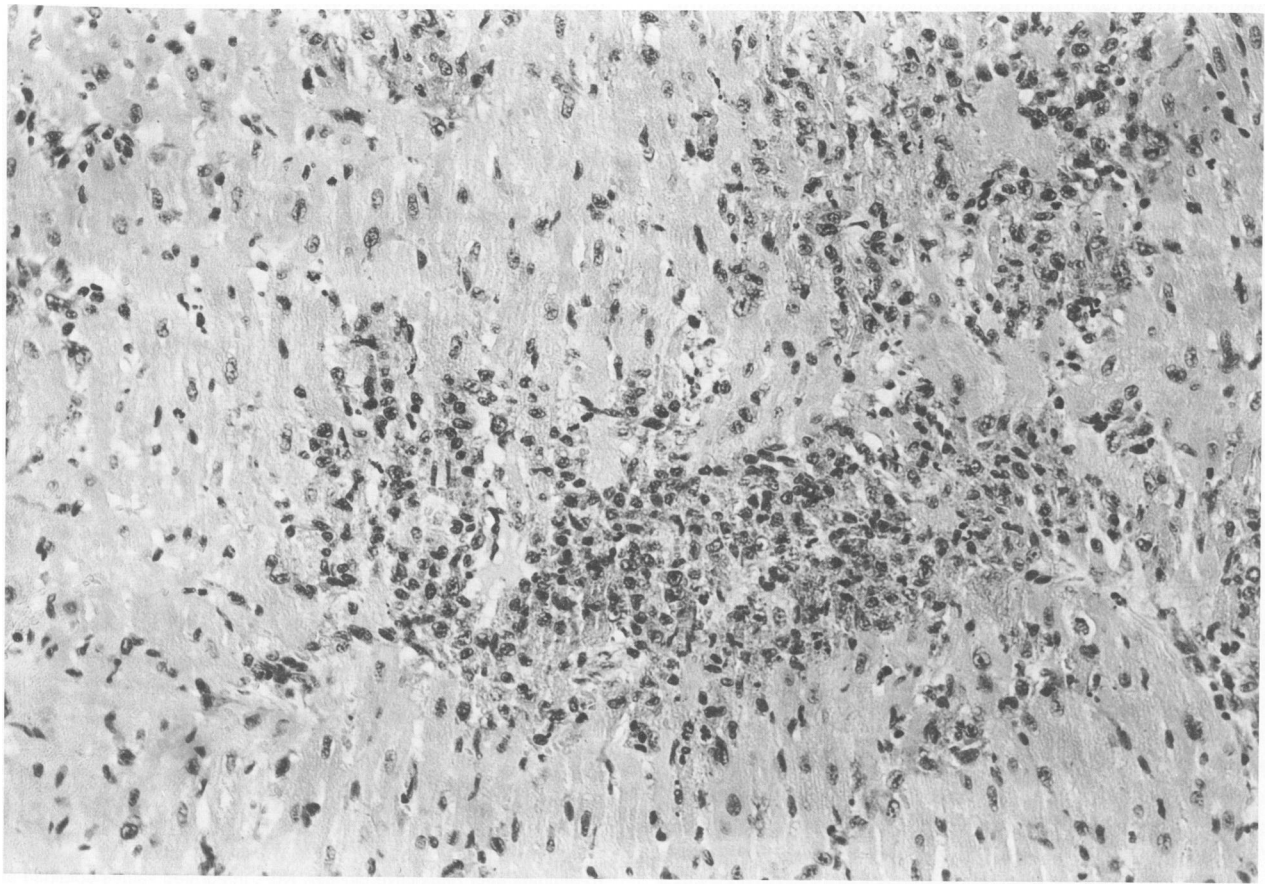

Figure 1—Histologic section of left ventricular myocardial from a rabbit killed 48 hours after NE infusion (2 μg/kg/min, 90 minutes). There is pronounced leukocytic infiltration and myofiber necrosis characteristic lesions scored 2.0 (H&E, × 125)

Table 1—Effect of Prazosin and Yohimbine on Arterial Blood Pressure and Heart Rate

Dose (μg/kg)	n	HR (bpm)	Arterial pressure (mmHg) Systolic	Diastolic	Mean
C		271 ± 8	122.5 ± 1.6	79.2 ± 2.2	92.5 ± 2.0
PZS_{50}	6	295 ± 8	103.3 ± 1.6*	62.5 ± 2.2*	74.2 ± 2.0*
C		231 ± 10	117.5 ± 3.5	80.0 ± 2.0	90.8 ± 2.5
PZS_{100}	6	307 ± 16*	105.8 ± 4.0	60.8 ± 2.7†	75.8 ± 2.6*
C		218 ± 7	108.8 ± 6.5	72.5 ± 2.7	83.8 ± 4.5
PZS_{200}	5	280 ± 13†	93.8 ± 1.1	61.3 ± 2.1†	72.5 ± 2.2†
C		261 ± 13	110.0 ± 2.5	73.8 ± 2.5	85.0 ± 2.5
$YHB_{2.5}$	5	311 ± 13†	108.1 ± 6.1	70.0 ± 4.7	76.9 ± 5.6
C		233 ± 15	123.6 ± 4.5	79.3 ± 3.6	90.0 ± 4.3
$YHB_{5.0}$	7	279 ± 13†	120.7 ± 6.7	75.7 ± 5.1	88.6 ± 4.5

Values are means ± SE. n, number of animals', HR, heart rate; C, control. $PZS_{50,100,200}$, prazosin, 50, 100, or 200 μg/kg, respectively; $YHB_{2.5,5.0}$, yohimbine, 2.5 or 5.0 mg/kg, respectively.

* $P < 0.001$.

† $P < 0.05$.

Results

Effects of Prazosin and Yohimbine on Basal Arterial Blood Pressure and Heart Rate

Arterial blood pressure and heart rate changes 15 minutes after administration of various concentrations of prazosin and yohimbine are summarized in Table 1. As expected, prazosin elicited significant reduction of both diastolic and mean arterial blood pressure at all three doses studied. The maximal depressor effect was reached at 50 μg/kg. In general, the systolic pressure was also decreased after prazosin treatment, but this reduction was not significant at the 100- and 200-μg/kg doses. This may be attributed to the significant elevation of heart rate.

The mean values for arterial blood pressure were lower after yohimbine administration, but the differences were not significant. However, yohimbine did elicit significant tachycardia at both the 2.5 mg/kg dose and 5.0 mg/kg dose.

Effects of Prazosin and Yohimbine on NE-Induced Hemodynamic and Metabolic Responses

Table 2 shows mean values of integrated mean arterial blood pressure and heart rate product during the 90-minute NE infusion period for all groups. The difference in changes of MABP × HR during NE infusion was not significant among the groups. Thus, it appears that the hemodynamically induced metabolic demand of the heart after NE infusion is probably significantly altered by the administration of α-adrenergic blockers. This concept is consistent with other studies.[12,33]

As expected, mean arterial glucose concentration rose progressively in NE-control animals from 120 to 285 mg/dl after 90 minutes of NE infusion. In contrast, the presence of the α_2-adrenergic receptor blocker yohimbine effectively prevented the NE-induced hyperglycemic effect. The hyperglycemic action of NE was not prevented by α_1-adrenergic blockade with prazosin.

No significant measurable changes of arterial pH and blood gases occurred during NE infusion in any experimental group.

Production of NE-Induced Cardiomyopathy in Rabbits

The NE-induced cardiomyopathy model in rabbits is well established in our laboratory.[12-14] A spontaneous, possibly viral, myocarditis is also known to occur in the rabbit.[2] For this reason, a series of control studies was conducted through the course of this study, and was used as an internal check for assessment of the reliability of histologic scores in the experimental groups. A comparison of mean histologic score of myocardial lesions in various groups is shown in Table 3. Eighteen of 23 control hearts (9 saline-infused controls and 14 NE-infused animals killed shortly after completion of

Table 2—Mean Arterial Blood Pressure Times Heart Rate Values (MABP × HR)

Group	n	MABP × HR Control	NE*
Saline + NE_2	12	24,611 ± 1,364	21,313 ± 1,409
PZS_{50} + NE_2	6	24,070 ± 1,940	22,288 ± 1,026
PZS_{100} + NE_2	6	22,436 ± 1,460	20,910 ± 873
PZS_{200} + NE_2	5	20,200 ± 1,460	18,738 ± 1,320
$YHB_{2.5}$ + NE_2	5	23,691 ± 2,713	23,025 ± 2,182
$YHB_{5.0}$ + NE_2	7	24,979 ± 2,377	27,180 ± 2,307

Values are means ± SE. n, number of animals; Control, values obtained 15 minutes after respective blocker; NE*, integrated values obtained at 10-minute intervals during 90 minutes of NE infusion, NE_2, infusion of NE at 2 μg/kg/min; $PZS_{50,100,200}$, prazosin, 50, 100, or 200 μg/kg, respectively; $YHB_{2.5,5.0}$, yohimbine, 2.5 or 5.0 mg/kg, respectively.

Table 3—Histologic Scores

Group	n	Time* (hours)	Histologic score (units)
Saline	9	48	0.17 ± 0.11
NE_2	8	2	0.25 ± 0.13
NE_2	6	6	0.17 ± 0.11
NE_2	12	48	1.63 ± 0.10

Values are means ± SE. n, number of animals; time*, time following infusion; NE_2, infusion of NE at 2 μg/kg/min.

infusion controls) showed no identifiable myocardial lesions. Only 2 hearts showed evidence of sparse myocardial injury (histologic score of 1.0), and 3 hearts had minimum focal cellular responses (histologic score of 0.5). In contrast, 10 of 12 animals that received NE and were killed 48 hours later were found to have marked myocardial lesions (5 scored 1.5, and 5 scored 2.0). The myocardial lesions in rabbits 2 days after NE infusion, therefore, are a direct consequence of the NE-induced myocardial injury and can be used in quantifying that injury.

Effects of α-Adrenoceptor Blockade on NE-Induced Myocardial Injury

Figure 2 shows the mean histologic scores from hearts in groups given NE only (NE_2) or with various prior amounts of α_1-adrenergic blocker prazosin. All groups given NE with pretreatment of α_1-adrenergic blocker scored significantly lower than the group of animals that received NE infusion only (1.63 ± 0.10). These ranged from 1.20 (±0.11) in rabbits with 50 μg/kg prazosin pretreatment to 0.30 (±0.10) in those animals pretreated with 200 μg/kg prazosin. Those animals given the intermediate dose of prazosin (100 μg/kg) had a mean score of 0.92 (±0.18).

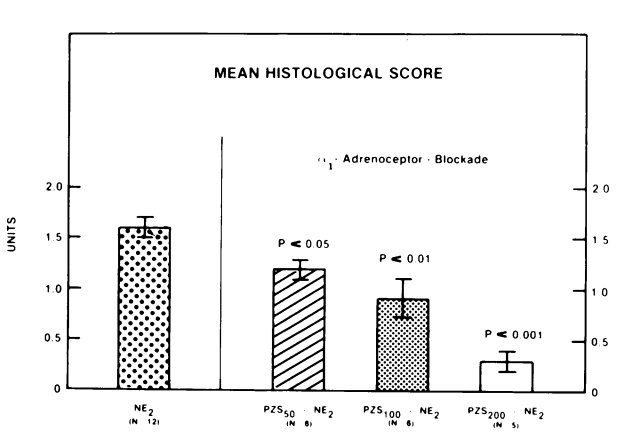

Figure 2—Histologic scores from hearts in groups given norepinephrine only (NE_2, 2 μg/kg/min) or prior adrenergic blockade with α_1-adrenoceptor blocker, prazosin (PZS, 50, 100, or 200 μg/kg). *N*, number of animals. Vertical brackets show SEM. *P* values are for comparison with the NE_2 group only.

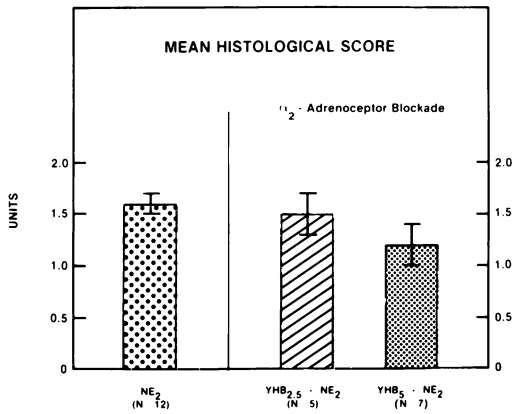

Figure 3—Histologic scores from hearts in groups given norepinephrine only (NE_2; 2 μg/kg/min) or prior adrenergic blockade with α_2-adrenoceptor blocker yohimbine (YHB; 2.5, 5.0 mg/kg). *N*, number of animals. *Vertical brackets* show SEM.

Figure 3 illustrates the effect of pretreatment with the α_2-adrenergic blocker yohimbine on NE-induced myocardial lesions. The data indicate that animals pretreated with yohimbine tended to show less cardiac injury, but the difference is not statistically significant.

Discussion

It is well recognized that catecholamines in large doses 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.[1,16] Impairment of intrinsic left ventricular function of hearts with catecholamine cardiomyopathy has also been identified.[14,17] The mechanism of the production of this type of cardiac injury is not yet known, although a number of hypotheses have been offered.[3,7,18-20]

The assumption of most previous studies was that catecholamine-induced cardiac lesions involved α-adrenergic pathways. Downing and Lee,[21] however, have found that α-adrenergic blockade with practolol or propranolol fails to significantly reduce cardiac injury induced by NE, but that α-adrenergic blockade with phentolamine, either alone or in combination with either one of the β-adrenergic blockers, markedly reduces myocardial lesions. Administration of the α-adrenergic agonist methoxamine produced a dose-related increase in the intensity of myocardial injury, morphologically identical to those resulting from norepinephrine. These findings are consistent with earlier observations in rats,[21] in which it was shown that beta blockade with propranolol prevented myocardial injury induced by isoproterenol (a specific β-adrenergic agonist) while having no effect on myocardial lesions caused by phenylephrine, a preferential alpha agonist. Furthermore, proprano-

lol pretreatment was only marginally effective against myocardial damage caused by epinephrine, whereas α-adrenergic blockade with phentolamine significantly reduced the development of myocardial necrosis.

In the present study we further delineated the contribution of α-adrenoceptor activation to the pathogenesis of NE-induced cardiomyopathy. Administration of prazosin (50–200 μg/kg), a highly selective α_1 adrenoceptor blocking agent, 15 minutes prior to the standard NE infusion (2 μg/kg/min) for 90 minutes significantly reduced myocardial lesion formation in a dose-related manner. This is reflected by the graded histologic scores in these hearts (Figure 2). In contrast, the presence of large doses of yohimbine (2.5–5.0 mg/kg), a relatively selective α_2-adrenoceptor blocking agent, was ineffective in preventing myocardial injury (Figure 3). These findings suggest that NE-induced cardiomyopathy probably results in large part from α_1-adrenoceptor-mediated mechanisms.

Alpha adrencoceptors have been classically grouped into two subtypes based on anatomic considerations with respect to adrenergic nerve synapses: α_1 for postsynaptic and α_2 for presynaptic.[22] The terms of α_1 and α_2 adrenoceptors should be used, therefore, from a pharmacologic,[23] functional,[24] or biochemical[25] point of view. Evidence is accumulating that α_2-adrenoceptors are located at both presynaptic and postsynaptic sites, whereas α_1-adrenoceptors seem to be exclusively located at postsynaptic sites.[26] At low doses yohimbine preferentially blocks presynaptic adrenergic receptors, which leads to release of endogenous NE by inhibition of the negative feedback control mechanism at the nerve terminals.[27] But at high doses (>0.3 mg/kg) yohimbine may block both subtypes of postsynaptic receptors.[24,27,28] This observation may explain the inability of yohimbine to potentiate the NE-induced myocardial injury as it would be expected.

It has been reported that stimulaton of α_1-receptors is not associated with changes in cyclic adenosine monophospate but results in alteration of cellular Ca^{2+} by increase influx of extracellular Ca^{2+} through a Ca^{2+} "gate" or "channel" in the plasma membrane or by release of bound intracellular Ca^{2+}. The precise molecular mechanisms are still unclear. Nevertheless, that α_1-adrenergic stimulation evokes cellular calcium accumulation has been demonstrated in many tissues, but not with α_2 activation.[19,29–32]

Glossmann and Horning[33] have found that D600 (calcium channel blocker) inhibits the binding of the antagonist prazosin to α-adrenoceptors in brain and heart membranes. The interaction of calcium channel blockers with α-adrenoceptors is competitive. Thus, it is therefore tempting to speculate that the α_1-adrenergic receptor blockers and calcium blockers may mediate through a common pathway.[29] In view of the foregoing consideration, it would seem reasonable to suggest that the differential effects of α_1 and α_2 on the protection of NE-induced myocardial damage in this study may be attributed to the calcium "channel" blocking properties of α_1-adrenoceptor blocker prazosin, which prevents intracellular calcium "overload" and preserves myocardium during excessive NE stimulation.

A second possibility is suggested by the more recent work of Simons and Downing,[34] who showed that NE-induced myocardial lesions may in part result from α-mediated coronary vasoconstriction action of NE and subsequently lead to myocardial ischemia and necrosis. The specific of α_1-adrenergic receptors are clearly demonstrated in coronary arteries.[35] However, the possibility of differential effects of two subtype α-adrenergic blocking agents on catecholamine-induced free radical formation cannot be ruled out.[20] All these considerations will clearly require further investigation to establish the precise cellular mechanism and to determine the significance of the excessive activation of the endogenous α-adrenergic system in cardiac regulation and pathology.

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