Contribution of α-Adrenoceptor Activation to the Pathogenesis of Norepinephrine Cardiomyopathy

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SUMMARY. Graded doses of norepinephrine and methoxamine were given to rabbits over a standard 90-minute infusion period to assess their potential for inducing myocardial injury. Lesions of myofiber necrosis and leukocytic infiltration were graded semiquantitatively in animals killed 2 days later. A close correlation was found between the dose of norepinephrine and the histological score (r = 0.912, P < 0.001). Mean arterial pressure rose from 100 mm Hg to a maximum of 129 mm Hg and averaged 115 mm Hg during infusion of 2 μg/min per kg. However, heart rate fell from 287 beats/min to average 208 beats/min. The pressure-rate product, an index of metabolic demand, showed no significant change and did not differ from saline-infused controls. β-adrenergic blockade with practolol (4 mg/kg) or propranolol (1 mg/kg) failed to significantly reduce cardiac injury with norepinephrine. However, α-adrenoceptor blockade with phentolamine (10 mg), alone or in combination with either of the β-antagonists, markedly reduced lesion formation as reflected by the histological score (P < 0.02). Administration of the α-agonist methoxamine produced dose-related increases in the intensity of myocardial injury (r = 0.938, P < 0.01), morphologically identical with those resulting from norepinephrine. Hemodynamic changes also were comparable. Phentolamine markedly reduced methoxamine injury. It may be concluded from these studies that norepinephrine cardiomyopathy results in large part from activation of the α-adrenergic system in the rabbit model.


In an earlier study, we demonstrated that a short-term (90-minute) infusion of norepinephrine (NE) given in relatively modest doses (2-3 μg/min per kg) elicits in the rabbit a consistent pattern of cardiac injury (Downing and Lee, 1978). This agrees with the previous findings of Schenk and Moss (1966), who studied a broad range of doses extended over periods varying from 1 to 15 hours. They found that the severity of myocardial damage is a function of both the dose and duration of NE infusion. To provide a more consistent and reproducible model, we have used a constant time base of 90 minutes. This is sufficient to produce extensive myofiber injury (Downing and Lee, 1978) readily identified by light microscopy and also by radionuclide cardiac imaging (Reeves et al., 1981). Moreover, measurements of cardiac function have revealed significant impairment of left ventricular performance when studied with afterload curves (Werner et al., 1980) or standard VF curves (Lee and Downing, 1982).

Whereas the aforementioned studies establish that administration of NE leads to substantial myofiber injury associated with reduced LV performance, the mechanism of injury has not been identified. We showed previously in studies with the isolated muscle preparation as well as the intact swine heart that insulin substantially reduces contractility responses to NE (Lee and Downing, 1976; Nudel et al., 1978). Similarly, insulin was found to reduce significantly the extent of myofiber injury when rabbits were infused with NE (Downing and Lee, 1978). These observations suggest a relationship between inotropic stimulation and catecholamine cardiomyopathy. In the present study, specific β- and α-antagonists and agonists were employed so that we might assess the receptor system predominantly involved in the pathogenesis. We were prompted by the fact that NE possesses both α- and β-stimulating properties, and by recognition of the fact that the α-agonist, methoxamine, elicits positive myocardial inotropic responses in several species (Nakashima et al., 1973; Endoh and Schumann, 1975; Rabinowitz et al., 1975; Lee et al., 1982). Dose-response relationships were established by means of a semiquantitative histological measure of cardiac injury. Hemodynamic changes presumed to reflect myocardial metabolic demand (Sarnoff et al., 1965, Rooke and Feigl, 1982) also were evaluated. Our findings indicate that the α-adrenergic system is an important pathogenetic factor in the rabbit.

Methods

Data to be presented in this study were obtained from 112 New Zealand white rabbits. All animals were anesthetized with pentobarbital, 30 mg/kg, and polyethylene catheters were placed in a femoral artery and vein. Arterial pressure was measured continuously with a Sanborn transducer, and heart rate was determined with a Sanborn cardiochometer. The latter was verified by manual assessment of pulse frequency from pressure traces inscribed by
Norepinephrine Injury
Morphological Patterns Associated with

Differences were considered significant when P < 0.05. Analysis of variance was performed for multiple group comparisons. Fisher's least significant difference test then was applied to assess the difference of individual mean values. Values for each of the two sections were averaged and used in scoring a given heart.

After infusion, the catheters were removed, the femoral wound surgically closed, and the animals returned to their cages after recovery from anesthesia. They were fed a standard diet and water ad libitum. All animals were killed 2 days later by cervical disarticulation or an overdose of pentobarbital via an ear vein. The hearts were immediately removed, emptied, and weighed. The atria and RV free wall were dissected and weighed, and the LV (+septum) separately weighed. Transverse "ring" sections of LV were obtained from the basal and mid-portions and fixed in 10% buffered formalin. They were prepared by standard histological methods and stained with hematoxylin and eosin for subsequent analysis.

Morphological evaluation employed a semiquantitative histological scoring system described previously (Downing and Lee, 1978). In brief, each section was graded by two observers according to the extent and intensity of the leukocytic response, without prior knowledge of the procedures used in a given animal. A maximum score of 2.0 was given when the lesions were florid, extensive, and transmural. Those with definite but sparse lesions were scored 1.0. Equivocal focal lesions were scored 0.5. Those judged to manifest injury more extensive than 1.0, but less than 2.0 (e.g., nontransmural) were assigned a score of 1.5. A score of 0 was given when no histological abnormality was present. Values for each of the two sections were averaged and used in scoring a given heart.

Substantial alterations in arterial pressure and heart rate occurred during catecholamine administration. Maximal changes and integrated mean values were determined from data obtained at 10-minute intervals. The pressure-rate product was calculated as an index of metabolic demand (Sarnoff et al., 1965; Rooke and Feggl, 1982). One-way analysis of variance was performed for multiple group comparisons. Fisher's least significant difference test then was applied to assess the difference of individual mean values. Student's t-test (Snedecor and Cochran, 1967) was used when two groups of unpaired data were compared. Differences were considered significant when P < 0.05.

Results

Morphological Patterns Associated with Norepinephrine Injury

The characteristic pattern of myocardial injury resulting from infusion of larger doses of NE is illustrated in Figure 1. There was a heavy cellular infiltrate of predominantly mononuclear cells in which large histiocytic cells were most numerous, accompanied by less frequent lymphocytes. Granulocytes, including eosinophils, occasionally were present, but only in small numbers. The infiltrate was largely interstitial, and tended to concentrate in association with foci of myofiber necrosis. In addition to fragmentation and focal myofiber destruction, numerous contraction bands and zones of granularity consistent with swollen mitochondria also were evident with light microscopy (Fig. 1). Z-lines were generally indistinct, and myofiber nuclei often were lost in the more active inflammatory foci. These changes were in general most pronounced in the papillary muscles and inner half of the ventricular wall. However, a transmural distribution often was observed in hearts subjected to higher doses of NE. There was no clear distinction between the intensity of free wall or septal involvement. Neither the larger coronary arteries nor myocardial arterioles exhibited discernible histopathological changes. Thrombi were never encountered in the more than 200 sections examined.

The mean histological scores obtained from rabbits infused with various amounts of NE (shown in Figure 2) illustrate the dose-response relationship. These ranged from 1.93 (±0.07) in animals given 3 μg/kg per min (NE3) to 1.34 (±0.19) in those given 1 μg/kg per min. Those given the intermediate dose (NE2) exhibited a mean score of 1.69 (±0.17). Animals infused with saline (NE0) showed no definite lesions (mean score, 0.06 ± 0.02). Regression analysis revealed a correlation coefficient of 0.912 (P < 0.001). All groups given NE scored higher than controls (P < 0.001). NE3 differed significantly from NE1 (P < 0.01), although the differences between these groups and NE0 did not reach statistical significance.

Hemodynamic Correlates

Arterial pressure and heart rate changes which occurred during infusion of various concentrations of NE are summarized in Figure 3 and Table 1. Initial values for mean arterial pressure averaged about 95 mm Hg, and for heart rate, about 290 beats/min. Saline infusion elicited no significant changes in either value (Table 1). However, infusion of progressively larger doses of NE elicited greater increases in both the maximal rise in arterial pressure and the average values measured for the 90-minute infusion period. These pressure changes also were accompanied by progressively greater reductions in heart rate.

Mean data from 12 rabbits infused with NE, 2 μg/min per kg, and the calculated pressure-rate (PXR) product are illustrated in Figure 3. Values obtained at 10-minute intervals throughout the 90-minute infusion period are shown. The control arterial pressure was 101 ± 2.9 mm Hg and rose to 128 ± 3.7 mm Hg 10 minutes after starting NE. The integrated mean arterial pressure was 115 ± 3.2 mm Hg during the 10- to 90-minute interval. Conversely, heart rate fell from 287 ± 7.9 to 210 ± 12.0 beats/min 10 minutes after starting NE. The integrated heart rate was 208 ± 9.5 beats/min. In the lower panel of Figure 3, the PXR
product calculated at each interval is compared with data from six saline-infused controls. Initial values averaged 29.0 (± 1.3) × 10^3 and 27.3 (± 2.4) × 10^3, respectively. At no point did the PXR product of the NE group rise or exceed values for the controls, and differences did not reach statistical significance. Thus it appears unlikely that excessive metabolic demand was a significant factor in the pathogenesis of the myocardial lesions identified in this group.

**Effects of Adrenoceptor Blockade**

So that further insight into the mechanism of NE-induced myofiber injury might be gained, selected β- and α-receptor-blocking agents were employed. Effects on the severity of myofiber injury as reflected by the histological score are shown in Figure 4, and corresponding hemodynamic data appear in Table 1. Analysis of variance was performed on the data shown in Figure 4 and gave a variance ratio (F) of 8.77 (P < 0.001). The Fisher test for individual group means showed that neither group subjected to β-blockade differed from that given norepinephrine only (NE2). Prior administration of a large dose (4 mg/kg) of practolol, a “pure” β1-blocking agent, was ineffective in reducing myofiber damage. The mean histological score (1.69 ± 0.10) was identical with that given the same dose of NE but without the β-blocking agent. Animals pretreated with propranolol, which has both β-blocking properties and an independent cardiac depressant action, tended to show less injury. The difference did not reach statistical significance, however.

In view of the well-known fact that NE is both a
β- and α-receptor agonist, a further series of animals were infused with NE after prior administration of the selective α-receptor-blocking agent, phentolamine (10 mg, total dose). As shown in Figure 4, when phentolamine was given in addition to practolol, the extent of myocardial injury was sharply reduced, as reflected by a histological score of 0.75 (±0.25) (P < 0.01). The same reduction in injury severity was observed with the combination of propranolol and phentolamine (P < 0.001). In view of the pronounced effect of α-blockade when combined with one of the β-blockers, a further series of nine animals was studied in which phentolamine alone was given prior to NE infusion. This appeared equally potent in reducing myocardial injury, and the mean histological score for this group was 0.87 (±0.15) (P < 0.02). Application of the Fisher test confirmed that all groups given phentolamine achieved significantly lower scores.

The effects of α-blockade may be contrasted with the absence of protection by hydrocortisone (100 mg) given prior to NE infusion in seven rabbits. The mean histological score (1.64 ± 0.23) was identical with animals given NE only. Potential membrane-stabilizing properties of this steroid were ineffective in preventing myocardial injury. This argues for a specific action of phentolamine through its α-receptor-blocking action.

Myofiber Injury following Methoxamine: Relation to Hemodynamic Changes

The foregoing studies suggest that α-adrenoceptor stimulation is a significant mechanism in the pathogenesis of myocardial injury by NE. To examine this hypothesis more directly, we studied the consequences of giving the alpha-agonist methoxamine. Representative photomicrographs from myocardial sections of a rabbit infused with methoxamine, 10 µg/min per kg for 90 minutes, are shown in Figure 5. The histological changes appear identical with those observed with NE. The pattern of myofiber damage and leukocytic infiltration was indistinguishable. No evidence for vascular injury or thrombus formation was identified. Accordingly, the same histological scoring system was applied, and the results from 25 animals are illustrated in Figure 6.

With the highest dose (15 µg/min per kg), the mean histological score was 1.70 ± 0.20. At the lowest dose (5 µg/min per kg), no significant injury occurred, but the intermediate dose produced a highly significant level of myocardial injury (P < 0.01). Moreover, pretreatment with phentolamine essentially eliminated the potential for cardiac injury observed with the highest dose of methoxamine. Thus, a pattern identical with that following NE resulted from α-
TABLE 1

<table>
<thead>
<tr>
<th>Arterial Pressure and Heart Rate Values during 90-Minute Infusion of Various Agents Indicated</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Max</td>
</tr>
<tr>
<td>Saline</td>
<td>92 ± 4.2</td>
<td>103 ± 6.4</td>
</tr>
<tr>
<td>NE₁</td>
<td>95 ± 3.7</td>
<td>112 ± 6.8</td>
</tr>
<tr>
<td>NE₂</td>
<td>101 ± 2.9</td>
<td>129 ± 3.4</td>
</tr>
<tr>
<td>NE₃</td>
<td>99 ± 3.1</td>
<td>135 ± 5.2</td>
</tr>
<tr>
<td>NE₂ + P₄</td>
<td>105 ± 4.6</td>
<td>139 ± 9.4</td>
</tr>
<tr>
<td>NE₂ + PPi + Rio</td>
<td>89 ± 3.1</td>
<td>124 ± 5.8</td>
</tr>
<tr>
<td>NE₂ + R₉</td>
<td>100 ± 2.4</td>
<td>113 ± 4.9</td>
</tr>
<tr>
<td>NE₂ + P₄ + R₉</td>
<td>101 ± 2.4</td>
<td>108 ± 3.2</td>
</tr>
<tr>
<td>NE₂ + PPi + R₉</td>
<td>72 ± 3.3</td>
<td>104 ± 3.9</td>
</tr>
<tr>
<td>M₅</td>
<td>106 ± 4.3</td>
<td>118 ± 5.4</td>
</tr>
<tr>
<td>M₁₀</td>
<td>99 ± 3.9</td>
<td>128 ± 4.7</td>
</tr>
<tr>
<td>M₁₅</td>
<td>96 ± 8.2</td>
<td>136 ± 11</td>
</tr>
<tr>
<td>M₁₅ + R₉</td>
<td>95 ± 7.9</td>
<td>88 ± 6.2</td>
</tr>
</tbody>
</table>

Max = greatest value recorded during infusion. Avg = average of values obtained at 10-minute intervals during infusion. n = number of animals. NE₁, NE₂, and NE₃ = infusion of norepinephrine at 1, 2, or 3 µg/min per kg, respectively. P₄ = practolol, 4 mg/kg. PPi = propranolol, 1 mg/kg. R₉ = Regitine (phentolamine), 10 mg. M₅, M₁₀, and M₁₅ = infusion of methoxamine at 5, 10, or 15 µg/min per kg, respectively. All agents given at rate of 0.382 ml/min.

Adrenergic activation with methoxamine, and this was prevented by giving the specific α-adrenoceptor-blocking agent, phentolamine.

Arterial pressure and heart rate changes with the three doses of methoxamine were virtually the same as those which occurred with the three doses of NE (Table 1). Phentolamine prevented the rise in arterial pressure and reduced the extent of cardiac slowing.

Discussion

This study confirms earlier investigations showing that administration of norepinephrine to the rabbit reproducibly elicits myofiber necrosis accompanied by an intense inflammatory response (Schenk and Moss, 1966; Downing and Lee, 1978). The extent of injury is a function of the dose of NE given (Fig. 2). Potential pathogenetic mechanisms have not been previously explored, however. NE is well known to elicit pronounced myocardial inotropic and chronotropic responses in all mammalian species examined. This is generally accompanied by substantial augmentation of cardiac metabolic demand (Sarnoff et al., 1965; Downing et al., 1973; Rooke and Feigl, 1982), raising the possibility of a supply-demand mismatch and ischemic injury to myocardium (Rona et al., 1963). Whereas this mechanism cannot be fully excluded in the present study, it seems unlikely on two counts. First, although arterial pressure increased, heart rate fell, and calculations of the pressure-rate product (PXR) (Sarnoff et al., 1965; Rooke and Feigl, 1982) suggest there was no increase in myocardial oxygen demand throughout the course of NE administration (Fig. 3; Table 1). Indeed, values for PXR tended to be lower than were found in saline-infused controls. Second, the histological pattern and leukocytic response differed from that expected with myofiber necrosis following an ischemic insult. In the
latter, a predominantly polymorphonuclear infiltrate would be anticipated. These cells were sparse or absent, and the infiltrate consisted largely of histiocytic mononuclear cells, perhaps cardiac histiocytes. Moreover, ischemia in which coronary flow does not remain interrupted frequently is accompanied by capillary damage and interstitial hemorrhage. These were never observed in the present or earlier study (Downing and Lee, 1978), nor was coronary vascular injury or thrombus formation identified by light microscopy. In view of these considerations, it would appear more likely that different factors are responsible.

It is clear that the $\beta$-receptor is the dominant adrenergic receptor associated with the cardiac cell (Furchgott, 1970; Watanabe et al., 1982). In most reported physiological studies, the $\beta$-blockers, practolol or propranolol, elicit nearly complete blockade of inotropic and chronotropic responses to usual test doses of isoproterenol or norepinephrine. A high level of block can be expected to persist for 2 hours or more. We employed these agents in the present study to test the hypothesis that myocardial injury by NE involved $\beta$-adrenergic pathways. As shown in Figure 4, neither practolol nor propranolol significantly altered the magnitude of lesion formation, as judged by the histological scoring system. This raised the possibility that NE causes direct injury to the myocyte, and possibly interstitial cells, independent of concurrent hemodynamic or metabolic changes (Ferrans, 1969). Indeed, myocardial lipid accumulation is one of the earliest biochemical changes in canine myocardium following catecholamine injury, and occurs with no reduction in coronary flow or its transmural distribution (Regan et al., 1966, 1971, 1972).

A second possibility is that $\alpha$-receptor stimulation may contribute to injury production by an agent (NE) with potent $\alpha$-activating properties (Watanabe et al., 1982). Our findings indicate that, in the rabbit, this is indeed a key pathway. Thus, when the $\alpha$-blocking agent, phentolamine, was given in addition to either practolol or propranolol, lesion production by NE was sharply reduced (Fig. 4). Moreover, myocardial injury was significantly reduced when phentolamine was given in the absence of $\beta$-blockade, and the mean histological scores did not differ among the three groups receiving phentolamine. It should not be inferred from these data that $\beta$-receptor activation plays no role in lesion production, because the histological scoring system would not be expected to discriminate small differences. Clearly, however, the dominant factor would likely be $\alpha$-activation. These findings are consistent with observations in isolated cat papillary muscle showing that phentolamine attenuates the increase in both force and adenylate cyclase activ-
increases of left ventricular contractility in the lamb, muscle and rat atrium (Rabinowitz et al., 1974, 1975). Oxamine in suitable concentrations elicits substantial consumption is provided by the demonstration that meth-
tors in myocardium. Further evidence for this as-

The hemodynamic data shown in Table 1 indicate increases in arterial pressure and reductions in heart rate comparable to the three doses of NE which were employed. Thus, as with NE, the calculated pressure-rate product did not increase during methoxamine infusion, rendering unlikely a significant change in myocardial metabolic demand sufficient to explain extensive myocardial injury.

Earlier findings that phenolamine reduced inotropic responses to agents with combined \( \alpha \)- and \( \beta \)-stimulating properties (NE, epinephrine, phenylephrine) in at least two species (Govier et al., 1966; Rabinowitz et al., 1974) suggest the presence of a physiologically significant concentration of \( \alpha \)-receptors in myocardium. Further evidence for this assumption is provided by the demonstration that methoxamine in suitable concentrations elicits substantial increases in force development in cat RV papillary muscle and rat atrium (Rabinowitz et al., 1974, 1975). Moreover, we have recently reported dose-related increases of left ventricular contractility in the lamb, as judged by changes in \( \frac{dp}{dt_{\text{max}}} \) and LV function curves (Lee et al., 1982). Maximal increases in \( \frac{dp}{dt_{\text{max}}} \) averaged about 20\%, however, substantially less than occurs with \( \beta \)-agonists. In isolated rabbit papillary muscle, the inotropic action of methoxamine is frequency dependent, the positive response being most pronounced at lower frequencies (Endoh and Schumann, 1975). In this regard, it is of interest that a significant bradycardia was observed during infusion of this agent in the present study (Table 1). Demonstration of the potential for transformation of myocardial \( \beta \)- to \( \alpha \)-receptors (Kunos and Nickerson, 1976; Kunos, 1977) suggests that the problem may be more complex than previously assumed, however.

Whereas there is substantial evidence for the existence of \( \alpha \)-receptors in cardiac muscle of several species, which, when activated, elicit inotropic changes, the precise pathway of inotropic stimulation is uncertain. It probably is not mediated by an increase in adenylate cyclase activity (Rabinowitz et al., 1974, 1975; Endoh and Schumann, 1975; Schumann et al., 1975). Moreover, in contrast with the \( \beta \)-adrenergic system, activation of \( \alpha \)-receptors induces little reduction of time-to-peak tension, and relaxation time is lengthened (Rabinowitz et al., 1975). These latter findings suggest that altered myocardial Ca++ translocation is a primary event. This hypothesis is also consistent with the reported frequency and temperature dependence of the \( \alpha \) system, and by the demonstration that the calcium channel blocker, D600, reduces positive inotropic responses to \( \alpha \)-stimulation (Endoh and Schumann, 1975; Endoh et al., 1975).

Recent evidence indicates that there exist two sub-
types of \( \alpha \)-receptors (Fain and García-Sainz, 1980; Hoffman and Lefkowitz, 1980). \( \alpha_1 \)-effects relate to phosphatidylinositol turnover with release of bound intracellular Ca++, as well as to increased uptake of extracellular Ca++ (Fain and García-Sainz, 1980). The relationship of these findings to \( \alpha \)-subtype distribution in myocardium is not known. However, it is of interest that an important mechanism leading to myofiber injury in ischemic heart disease involves accelerated degradation of membrane phospholipids (Chien et al., 1979). This is accompanied by marked increases in myocardial Ca++ concentration and a several-fold increase in passive Ca++ permeability of sarcoplasmic reticulum. A possible relationship between these observations and the myofiber injury resulting from methoxamine administration described in the present study is of course speculative. Determination of the significance of the \( \alpha \) system for cardiac regulation, or for endogenously generated myocardial damage will require further study.

We acknowledge the technical assistance of Ronald Gordon, Marilyn Palkowski, Sandra Rancourt, and Terry Zibello. Statistical assistance was provided by Dr. Colin White.

This work was supported in part by Grants HL 20401 and HL 08659 from the National Institutes of Health.

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INDEX TERMS: Rabbits • Methoxamine • Phentolamine • Propranolol • Myocarditis • Hydrocortisone • Cardiac morphology
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doi: 10.1161/01.RES.52.4.471

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