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. Ischemia or a supply-demand mismatch are unlikely mechanisms. We speculate that alterations in myofiber Ca++ translocation, uptake, and binding induced by α-receptor activation may contribute to membrane damage. (Circ Res 52: 471-478, 1983)

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 cardiohometer. 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 $P < 0.05$. Values for each of the two sections were averaged and used when two groups of unpaired data were compared. Student's t-test (Snedecor and Cochran, 1967) was applied to assess the difference of individual mean values. Fisher's least significant difference test then was applied to examine the dose-response relationship. These included practolol (Ayerst), 4 mg/kg; propranolol (Ayerst), 1 mg/kg; and phentolamine (Ciba-Geigy), 10 mg. An additional seven animals were prepared and given hydrocortisone (Sigma), 100 mg, 15 minutes prior to NE administration.

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 Feigl, 1982). One-way analysis of variance was performed for multiple group comparisons. Fisher's least significant difference test then was applied to examine 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 ($P < 0.001$). NE1 differed significantly from NE3 ($P < 0.01$), although the differences between these groups and NE2 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) x 10^3 and 27.3 (± 2.4) x 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 those given the same dose of NE but without the β-blocking agent. Animals pretreated with propranalol, 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

FIGURE 1. Representative histological section of left ventricular myocardium from a rabbit killed 48 hours after NE infusion (2 µg/min per kg; 90 min). There is extensive leukocytic infiltration, contraction band formation, and focal myofiber damage characteristic of lesions scored 2.0. H&E stain. Original magnification, 310X.
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

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<tr>
<th>Arterial Pressure and Heart Rate Values during 90-Minute Infusion of Various Agents Indicated</th>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<tr>
<td>Control</td>
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<tr>
<td>92 ± 4.2</td>
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<td>NE1</td>
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<td>M15</td>
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<td>M5 + RIO</td>
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</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. RIO = 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.

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 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, propranolol, or dobutamine, elicit nearly complete blockade of inotropic and chronotropic responses to usual test doses of isoproterenol or norepinephrine. A higher 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 propranolol nor dobutamine 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 propranolol or dobutamine, 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-
crease in arterial pressure and reductions in heart
The hemodynamic data shown in Table 1 indicate
alculating properties (NE, epinephrine, phenyleph-
ities induced by norepinephrine (Rabinowitz, 1974).
imilar findings have been reported for guinea pig
More compelling evidence that α-receptor stimu-
lation leads to myocardial injury is provided by the
results following methoxamine administration. As
illustrated in Figure 5, the morphological pattern of
jury was identical with that following NE infusion. The
histological score was a positive function of the
doze given (Fig. 6), and lesion production was essen-
tially absent in animals pretreated with phentolamine.
The hemodynamic data shown in Table 1 indicate
that the doses of methoxamine chosen elicited in-
creases 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 phentolamine reduced ino-
tropic responses to agents with combined α- and β-
stimulating properties (NE, epinephrine, phenyleph-
rine) in at least two species (Govier et al., 1966;
Rabinowitz et al., 1974) suggest the presence of a
physiologically significant concentration of α-recep-
tors in myocardium. Further evidence for this as-
sumption is provided by the demonstration that meth-
oxamine 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 $dP/dt_{max}$ and LV function
curves (Lee et al., 1982). Maximal increases in $dP/
$dt_{max}$ averaged about 20%, however, substantially less
than occurs with β-agonists. In isolated rabbit papil-
ary 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 infu-
sion of this agent in the present study (Table 1).
Demonstration of the potential for transformation of
myocardial β- to α-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 exist-
ence of α-receptors in cardiac muscle of several spe-
ies, which, when activated, elicit inotropic changes,
the precise pathway of inotropic stimulation is uncer-
tain. 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 β-adrenergic
system, activation of α-receptors induces little reduc-
tion of time-to-peak tension, and relaxation time is
lengthened (Rabinowitz et al., 1975). These latter find-
ings suggest that altered myocardial Ca ++ transloca-
tion is a primary event. This hypothesis is also con-
sistent with the reported frequency and temperature
dependence of the α system, and by the demonstra-
tion that the calcium channel blocker, D600, reduces
positive inotropic responses to α-stimulation (Endoh
and Schumann, 1975; Endoh et al., 1975).
Recent evidence indicates that there exist two sub-
types of α-receptors (Fain and García-Sáinz, 1980;
Hoffman and Lefkowitz, 1980). Alpha1-effects relate
to phosphatidylinositol turnover with release of
bound intracellular Ca ++, as well as to increased
uptake of extracellular Ca ++ (Fain and García-Sáinz,
1980). The relationship of these findings to α-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
sarcomplasmic reticulum. A possible relationship be-
tween these observations and the myofiber injury
resulting from methoxamine administration described
in the present study is of course speculative. Deter-
mination of the significance of the α-system for car-
diac regulation, or for endogenously generated myo-
cardial damage will require further study.

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