Mechanism of Norepinephrine Depletion in Experimental Heart Failure Produced by Aortic Constriction in the Guinea Pig

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Increasing evidence indicates that the activity of the sympathetic nervous system is altered in congestive heart failure. The stress of muscular exercise elevates the concentration of circulating norepinephrine to abnormally high levels in patients with chronic heart failure, and even at rest such patients exhibit evidence of augmented activity of the adrenergic nervous system, as reflected in an increased urinary excretion of norepinephrine. Biopsies of atrial tissue obtained from patients with heart failure have shown that the concentration of norepinephrine may be markedly reduced. To determine if depletion of the total cardiac store of norepinephrine is characteristic of congestive heart failure in general, it was necessary to study this problem in an experimental model. However, it is appreciated that no single experimental preparation can satisfactorily reproduce the various forms of heart failure encountered clinically. Accordingly, we have examined this problem in two mammalian species in which heart failure was produced by different interventions. In the dog, primary right heart failure was investigated while in the guinea pig, primary left heart failure was studied, as described in a preliminary communication. The present report consists of an analysis of the time course of the changes in cardiac norepinephrine concentration which occurred in the guinea pig, as well as studies on the mechanisms responsible for the alterations of norepinephrine stores.

Methods

Supravalvular aortic constriction was produced by a modification of the technique previously described by Gertler and Schwartz and Lee. Adult guinea pigs, weighing between 850 and 900 g, were operated upon under sterile conditions; intermittent positive pressure respiration was supplied by way of the pharynx with a small animal respirator after esophageal occlusion with a balloon catheter. The ascending aorta was constricted with a circular wire clip covered with polyethylene, the clip having a known internal diameter of 2.2, 2.0 or 1.9 mm. The lumina of the constricted portion of the aortas averaged 25, 10, and 5% of normal, respectively. Sham operations were performed in 15 animals by placing a nonconstricting clip (4.0 mm) on the aorta or by dissecting out the aorta, placing a 2.0 mm clip in the usual manner and immediately removing it.

The animals were sacrificed by a blow on the head at varying intervals of time, between 1 and 71 days after aortic constriction or sham operation. The hearts and kidneys were removed and frozen immediately in dry ice. The atria were dissected along the atrioventricular groove and the free wall of the right ventricle was removed, leaving the left ventricle and the interventricular septum as a unit. Unoperated control animals were sacrificed and the tissues were removed in a similar manner. Body weights were measured preoperatively and at the time of sacrifice, but all relations of heart weight to body weight were based on preoperative body weight measurements, although no consistent changes in body weight occurred postoperatively. In 12 animals portions of the lungs and liver were taken for pathologic examinations. In 30 animals, immediately prior to sacrifice, central venous pressure was measured utilizing a water manometer and a polyethylene catheter introduced into the superior vena cava through the jugular vein under local anesthesia.

In a total of 27 animals, 14 controls and 13 studied 10 days after aortic constriction with a 2.0 mm clip, the uptake and retention of infused

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l-norepinephrine were investigated. Thirty-three \( \mu g/kg/min \) l-norepinephrine was infused in a 4 ml volume of 5% dextrose and water for one hour through a catheter introduced via the jugular vein under local anesthesia. Animals were sacrificed immediately at the end of the infusion, and at various time intervals thereafter.

The net turnover rate of norepinephrine in the heart was studied in another group of 48 guinea pigs after labeling the tissue norepinephrine stores with a small dose of tritiated DL-norepinephrine. Twenty-four control animals, and 24 animals in which the aorta had been constricted with a 2.0 mm clip 10 days previously, were given an intravenous injection of 100 \( \mu c/kg \) of DL-norepinephrine-7-\( H_3 \), which had a specific activity of 58.8 \( \mu c/\mu g \), and the animals were sacrificed at various time intervals following injection.

Studies of the subcellular distribution of norepinephrine were done by differential centrifugation of a homogenate of the left ventricle using the technique described by Campos and Shide-man. The homogenate was centrifuged in the cold at 2000 \( \times \) g for five minutes and the supernatant fluid containing the contents of the cells ruptured by homogenization was separated from the pellet, which is considered to consist of intact cells. This initial supernatant fluid was then fractionated into a pellet of subcellular particles and a second supernatant portion containing the cell fluid, by centrifugation at 100,000 \( \times \) g for one hour. Norepinephrine was extracted from these three fractions with trichloroacetic acid and measured as described below.

Norepinephrine analyses were done only in animals sacrificed and not in those that died spontaneously. In all determinations the tissues were homogenized in the cold with 10 volumes of 5% trichloroacetic acid in a Virtis homogenizer. After centrifugation at 20,000 \( \times \) g at 4°C for 20 minutes, a clear supernatant was used for adsorption on aluminum oxide at pH 8.4. Catecholamines were eluted from the aluminum oxide with 0.2 N acetic acid and measured in an Amino-Bowman spectrophotofluorometer after oxidation to the trihydroxyindole with ferricyanide at pH 6.1. Readings were made at activation wave lengths of 390 and 425 \( \mu m \), and at fluorescence wave lengths of 500 and 525 \( \mu m \), which allowed for the differentiation of norepinephrine and epinephrine. The concentration of norepinephrine was determined, and from this value and the weight of the ventricle the total amount of norepinephrine in the ventricle was calculated and expressed per kilogram of body weight. Recoveries of norepinephrine added to tissue samples averaged 88.5% and the results presented were not corrected for this recovery. The standard deviation of the measurement was 0.06 \( \mu g/g \), based on 21 duplicate measurements. For determination of tritium labeled norepinephrine, a portion of the eluate from aluminum oxide was lyophilized, redissolved in 0.2 ml distilled water, mixed with 10 ml of counting solution and counted in a liquid scintillation spectrometer. Internal radioactive standards were used and the efficiency of the system was found to be 25%. Sufficient counts were obtained to maintain a standard deviation of counting below 1%. The standard deviation of this measurement of norepinephrine radioactivity in tissue, based on 11 duplicate measurements, was 0.15 \( \mu c/g \).

Results

I. EVIDENCE OF HEART FAILURE

The mortality for the period from six hours to ten days after operation was determined in a total of 144 animals. None of the 15 sham-operated animals died, but 28% of the 22 animals with a 2.2 mm diameter aortic clip died, as did 49% of the 98 animals with a 2.0 mm diameter aortic clip, and all 9 animals with a 1.9 mm clip. In the animals that were not sacrificed, but died as a result of the constriction, death was attributed to heart failure. The lungs were frothy and edematous; varying degrees of fluid accumulation were noted in the pleural and abdominal cavities; and the liver was usually congested as well. Congestion of the lungs and liver were observed on microscopic examination. Similar pathologic changes were observed in the animals sacrificed for norepinephrine analysis following constriction of the aorta.

In 11 control animals the central venous pressure, referred to the level of the right atrium, averaged \(-8 \pm 2 \) (sem) mm H\(_2\)O. This pressure was elevated significantly in animals constricted with a 2.0 mm clip, both in the 14 guinea pigs studied 10 days postoperatively (avg = +19 ± 10 mm H\(_2\)O, \( P < 0.01 \)), and in the 5 animals studied 52 to 65 days postoperatively (avg = +44 ± 10 mm H\(_2\)O, \( P < 0.01 \)).

II. VENTRICULAR WEIGHTS

The left ventricular weights in 19 unoperated control animals averaged 1.80 ± 0.03 g/kg. In 15 sham-operated guinea pigs, sacrificed 3 to 71 days after operation, the left ventricular weight was not significantly different, and
averaged 1.77 ± 0.04 g/kg. Following constriction with the 2.0 mm clips the left ventricular weight was increased significantly above control, by an average of 33% at 3 days ($P < 0.01$) and thereafter it increased more slowly, reaching an average of 88% above control at 65 days ($P < 0.01$, fig. 1). Following constriction with the 2.2 mm clips the changes were similar, though of a somewhat smaller magnitude, during the first 20 days after constriction (fig. 1).

The right ventricular weights in 19 unoperated animals averaged 0.50 ± 0.01 g/kg. In 36 guinea pigs constricted with the 2.0 mm clips and sacrificed at various times after operation this value was elevated slightly, but significantly ($P < 0.02$), averaging 0.60 ± 0.03 g/kg, but the time course of this change was not as consistent as in the left ventricle. No change in right ventricular weight occurred in the 16 animals constricted with the 2.2 mm clips (avg = 0.50 ± 0.02 g/kg).

### III. Cardiac Norepinephrine Concentration and Content

In 15 normal guinea pigs the left ventricular norepinephrine concentration and content averaged 1.82 ± 0.10 µg/g and 3.01 ± 0.17 µg/kg body weight respectively, while in the right ventricle the corresponding values were 2.15 ± 0.12 µg/g and 0.93 ± 0.04 µg/kg. Twenty-four hours after constriction no changes in norepinephrine concentration or content were observed. However, 5 days following constriction with the 2.0 mm clips the right and left ventricular concentrations of norepinephrine had fallen markedly to values which averaged 22 and 24% of control respectively. In the left ventricle there was some variation with time but the levels remained depressed for 65 days (fig. 2A), while in the right ventricle the concentration was lowest 5 days postoperatively (fig. 2B). The total ventricular content of norepinephrine also declined; in the left ventricle this value reached
its lowest level at 5 days, at which time it averaged 36% of control (fig. 2C). Thereafter it rose to values which approached the control 65 days postoperatively. The depression of right ventricular norepinephrine content was also most marked 5 days postoperatively and then rose to reach control levels at 50 days (fig. 2D). The norepinephrine contents of the 15 sham-operated animals, sacrificed 3 to 71 days after operation, averaged 2.57 ± 0.17 and 0.85 ± 0.06 respectively in the left and right ventricles. Although these values were slightly lower than those observed in the control animals the differences were not significant.

In considering only the values at 10 days after operation it was observed that the left ventricular norepinephrine concentrations and contents, 0.48 ± 0.08 µg/g and 1.20 ± 0.12 µg/kg, in 8 animals constricted with a 2.0 mm clip, were both significantly lower than those values obtained in the 6 sham-operated animals, 1.36 ± 0.15 µg/g and 2.33 ± 0.24 µg/kg (P < 0.01). The values in animals with aortic constriction were also significantly lower than those observed in control animals (P < 0.01). Five of the 8 animals with aortic constriction had norepinephrine concentrations and 4 had norepinephrine contents which were below the 95% confidence limits established for the 6 sham-operated guinea pigs. In view of this statistically significant difference in the values obtained in the sham-operated animals 10 days after operation and those constricted with a 2.0 mm clip 10 days postoperatively, all subsequent studies on the mechanism of norepinephrine depletion were confined to animals whose aortas had been constricted with 2.0 mm clips 10 days previously.
The norepinephrine contents in both ventricles of 16 animals with 2.2 mm clips, and sacrificed at various time intervals (5 to 36 days) thereafter, were depressed significantly below the control ($P < 0.01$). These values averaged $2.19 \pm 0.18$ and $0.74 \pm 0.04$ $\mu$g/kg respectively in the left and right ventricles. However, the values for left ventricular norepinephrine content in the animals constricted with 2.0 mm clips and sacrificed 10 days postoperatively were significantly ($P < 0.05$) lower than those obtained from the animals constricted with 2.2 mm clips 10 days previously.

The hearts obtained from control animals contained amounts of epinephrine which were less than 10% of the norepinephrine present, values which were so low that the accuracy of the determination does not justify quantitative presentation of the data. However, no increases in cardiac epinephrine were observed in the tissues obtained from the animals with heart failure.

IV. RENAL NOREPINEPHRINE CONCENTRATION

The concentration of norepinephrine in the kidneys of 11 control animals averaged $0.53 \pm 0.05$ $\mu$g/g. In the 31 animals sacrificed 5 to 65 days after aortic constriction with the 2.0 mm clips the concentration of norepinephrine was not consistently depressed at any time after operation and averaged $0.53 \pm 0.04$ $\mu$g/g. However, the renal norepinephrine concentration in 3 guinea pigs with aortic constriction was less than the lowest normal value ($0.32 \mu$g/g). These animals exhibited particularly severe heart failure and in all 3 of them there was a striking reduction of the left ventricular norepinephrine concentration to less than 0.32 $\mu$g/g.

V. EFFECTS OF NOREPINEPHRINE INFUSIONS

The elevations of norepinephrine concentrations in the hearts and kidneys produced by the infusion of norepinephrine in 14 control animals and in 13 guinea pigs, in which the aorta had been constricted with 2.0 mm clips 10 days previously, are shown in figure 3. In the normal animals the left and right ventricular, and renal, concentrations of norepinephrine rose to peak values at the completion of the infusion and thereafter the

![Figure 3](http://circres.ahajournals.org/)

**FIGURE 3**

Effects of infusion of norepinephrine (NE) on the concentrations of norepinephrine in the left ventricles (A), right ventricles (B), and kidneys (C) of normal guinea pigs (solid lines and circles) and guinea pigs with congestive heart failure (CHF, open circles and broken lines). Vertical bars represent $\pm 1$ SEM. Stippled areas represent duration of infusion and the numbers in parentheses refer to the number of animals in each group sacrificed at the various times.

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concentrations declined over the ensuing three hours to values which approached the controls. In contrast, the increase in the ventricular concentrations in the animals with aortic constriction was minimal whereas the renal norepinephrine in this group increased in a manner similar to that observed in the normal group. At 30 minutes following completion of the infusion in the normal animals the left ventricular norepinephrine concentration had increased by 1.16 µg/g, an increment above control which was significantly greater than that noted in animals with aortic constriction, 0.32 µg/g (P < 0.01). Similarly, in the right ventricle the augmentation of norepinephrine concentration observed 30 minutes after completion of the infusion was greater in the normal animals than in those with aortic constriction. Thirty minutes following the infusion the plasma norepinephrine concentrations were comparable in the two groups, averaging 0.028 ± 0.011 µg/ml in the control animals and 0.033 ± 0.014 µg/ml in the constricted animals.

VI. LEFT VENTRICULAR NOREPINEPHRINE NET TURNOVER RATE

One hour after injection of tritium-labeled DL-norepinephrine, the left ventricles of the normal animals contained an average of 0.76 ± 0.01 µc/g, while this value was only 0.37 ± 0.05 µc/g in the animals with aortic constriction. Thereafter, the absolute levels of radioactivity declined at the same relative rates in both the normal guinea pigs and those with aortic constriction, these declines being relatively rapid initially and slower after 24 hours. When expressed in terms of specific activity one hour after injection, the values in the control animals averaged 3.04 × 10⁻¹ ± 0.04 × 10⁻¹ µc/g, while in the heart failure group they averaged 4.92 × 10⁻¹ ± 0.75 × 10⁻¹ µc/g. Thereafter, no consistent differences were found and the values obtained from the two groups exhibited the same disappearance slopes with two exponential components, the half-lives of which equaled 5 and 19 hours respectively (fig. 4).

VII. SUBCELLULAR DISTRIBUTION OF NOREPINEPHRINE

In seven control animals differential centrifugation of a homogenate of left ventricle revealed that the coarse fraction, which is considered to represent predominantly unbroken cells, contained an average value of norepinephrine of 0.58 ± 0.07 µg/g (40% of the total). The particulate and soluble fractions of the ruptured cell contents were found to contain averages of 0.37 ± 0.03 µg/g (40%) and 0.29 ± 0.02 µg/g (20%) respectively. In the six animals with aortic constriction, the norepinephrine contained within each of these three fractions was 0.26 ± 0.06 µg/g (45%), 0.18 ± 0.04 µg/g (32%), and 0.13 ± 0.04 µg/g (23%) respectively. Thus, in the animals with aortic constriction the norepinephrine contained in each of these fractions was reduced markedly in absolute terms, but there was no striking alteration of the subcellular distribution (table 1).

Discussion

Severe constriction of the ascending aorta of the guinea pig results in a clinical picture which resembles that observed in human congestive heart failure. The animals appear dyspneic and have pulmonary rales, enlarged palpable livers, and elevated central venous pressures. At autopsy their lungs are severely congested. When this relatively acute type of heart failure is produced the concentrations of norepinephrine in both the left and right ventricles decline. These findings agree with observations on biopsies of atrial appendages and left ventricular papillary muscles in patients with heart failure, as well as with the measurements of norepinephrine in cardiac tissues of dogs with experimental heart failure. It is interesting that reductions of norepinephrine concentration occurred in the present investigation, in which the primary hemodynamic burden involved the left ventricle, as well as in the dogs in which the abnormal load was placed on the right ventricle by creating pulmonary stenosis and tricuspid regurgitation. Furthermore, although the reductions of norepinephrine concentration were more striking in the left ventricles of the guinea pigs, significant reductions occurred also in the right ventricles. Supravalvular
aortic constriction in the guinea pig is characterized by the development of considerable ventricular hypertrophy. Nonetheless, the total norepinephrine contents in the left ventricles following aortic constriction by the 2.0 mm clip were found to be diminished significantly below the values observed in the sham-operated animals, and the marked reductions in norepinephrine concentrations therefore cannot be attributed solely to the dilution of an unchanged number of nerve endings, each with a normal complement of norepinephrine, in an increased muscle mass.

The preparation utilized in this study allowed
an examination of both the time course of norepinephrine depletion and an evaluation of the manner in which the severity of the hemodynamic burden affected this phenomenon. The reductions of norepinephrine concentration and content occurred rapidly, and were already maximal five days after constriction of the aorta, at a time when substantial left ventricular hypertrophy had also occurred. In following the time course of these changes it was noted that while the left ventricles continued to increase in weight, the concentration of norepinephrine did not decline further, and the total content of norepinephrine in this chamber gradually increased. Furthermore, it was observed that the extent of the reduction of norepinephrine store was related to the severity of the aortic constriction. In the interpretation of the time course of norepinephrine depletion it is necessary to consider that the measurements were made only in the animals which were sacrificed, and not those which died in heart failure. As a result many of the sickest animals were automatically excluded. The possibility must also be considered that the norepinephrine depletion is related to the stress of the operative procedure and the acute hemodynamic burden imposed on the heart. However, this is unlikely in view of the absence of depletion observed on the first postoperative day (fig. 2).

In order to determine whether the observed alterations of norepinephrine stores in the heart also involved adrenergic nerves in other tissues, the norepinephrine concentration of the kidney was studied. In contrast to the large and consistent changes observed in the heart no significant depletion of renal stores of norepinephrine occurred in the failure group as a whole. Thus, it is clear that the reduction of norepinephrine store is not uniform in all sympathetic nerve endings. However, it is possible that norepinephrine depletion may occur in noncardiac nerves since, in an occasional guinea pig with heart failure, decreases in renal norepinephrine concentration accompanied profound depression of cardiac stores.

The uptake and retention of exogenous norepinephrine were studied in an attempt to elucidate the mechanisms responsible for the alterations in cardiac norepinephrine stores in heart failure. In the normal guinea pig large doses of norepinephrine resulted in substantial increases of the cardiac norepinephrine stores. In the animals with heart failure the same infusion resulted in smaller increments, and the peak levels observed in the heart were not only much lower than the peak levels produced by the infusion in normal guinea pigs, but were even lower than the control values seen in the normal group. However, the increase in left ventricular norepinephrine stores was roughly proportional to the absolute concentration present in each group prior to the infusion. These findings could be explained by: 1) an increase in the net turnover (release and/or breakdown) of intraneuronal norepinephrine stores, 2) the presence of a normal total number of nerve endings, each of which is defective in its ability to take up and/or retain norepinephrine, 3) a reduction in the total number of nerve endings, each of those left retaining a normal ability to take up and/or retain norepinephrine, and 4) a combination of these mechanisms.

To investigate these possibilities a smaller dose of radioactive norepinephrine was administered and its turnover was determined by following the specific activity in the left ventricle for 72 hours. In the animals with heart failure, the amount of radioactive norepinephrine present in the tissue one hour after injection was significantly less than that present in the normal group, thus extending to much smaller doses the findings observed with infusions of large quantities of unlabeled norepinephrine. In both groups of animals the decline in specific activity was complex, and exhibited two exponential components. This finding is similar to the observations of other investigators in the hearts of several mammalian species and is compatible with the presence of a multicompartmental distribution of norepinephrine. The absolute levels of specific activity and the rates of disappearance were essentially identical in the normal animals and those with heart failure, indicating that the relative net turnover rates were the
same, although the absolute net turnover rates were reduced in the animals with heart failure. Thus, in the animals with failure, the smaller increment of norepinephrine in the hearts after infusion cannot be explained by a more rapid net turnover of norepinephrine, but must be interpreted as an abnormality of uptake and/or retention and it is likely that this defect is responsible for the observed depletion of norepinephrine stores. In view of the smaller norepinephrine stores, the presence of a normal net turnover rate also indicates that the rate of formation and the release and destruction in the left ventricle must actually be reduced in the heart failure group.

Although the data obtained do not provide a definitive answer to the question of whether the underlying defect is a simple reduction of the number of nerve endings or a quantitative abnormality in the function of a normal complement of nerves, two findings support the former hypothesis. First, the close similarity of the two components of the specific activity decay curves suggests that the compartmentalization and the rate of distribution and handling of the label within these compartments is similar in the hearts of the normal animals and those with heart failure. Second, the subcellular distribution of norepinephrine was also similar in the two groups, with approximately the same fractions of the total norepinephrine present in particle-bound and soluble components. It seems unlikely that a nerve, whose function has been altered sufficiently to deplete it of norepinephrine, would not manifest some alterations in those properties. Thus, these findings are more compatible with the presence of a reduced number of normal nerve endings than with a fundamental abnormality in the function of all nerve endings. However, the possibility cannot be completely excluded that a normal complement of nerves is present, operating with a small but active store of norepinephrine while the content in a larger, less active compartment is reduced.

A number of biochemical abnormalities have been observed in the hearts of patients or experimental animals with heart failure.\textsuperscript{7, 10-18} It has always been difficult to determine whether any of these chemical changes are the underlying events responsible for the abnormality of myocardial performance or whether they are secondary. There is no reason to believe that the observed alterations in cardiac norepinephrine are primary. In view of the powerful inotropic effects of sympathetic nerve activity, the adrenergic system offers a potential mechanism for augmenting cardiac function when the latter is depressed. However, the observed alterations in the total amount and retaining capacity of norepinephrine in the heart suggest that sympathetic nerve function may be abnormal in heart failure. It is possible that the depletion of norepinephrine stores may result in a reduction of the quantity of this substance released per nerve impulse and this disturbance may diminish the sympathetic support to the failing heart, thereby intensifying any heart failure that exists.

**Summary**

The present study was undertaken to evaluate the influence of heart failure on the cardiac stores of norepinephrine, and to elucidate the mechanisms responsible for the changes observed. Congestive heart failure was produced in the guinea pig by supravalvular aortic constriction. Significant reductions in both the concentration and content of norepinephrine in the ventricles were observed, the magnitude of changes being related to the severity of the constriction. The renal concentration of norepinephrine was not usually affected. Infusions of large quantities of norepinephrine produced elevations of ventricular norepinephrine concentrations which were significantly less in guinea pigs with heart failure than in normal animals. Injections of lesser quantities of radioactive norepinephrine also resulted in smaller amounts of this material in the hearts of animals with failure. Measurement of the decay of specific activity indicated that heart failure did not alter the net turnover of norepinephrine in the left ventricle. From these findings it has been concluded that a defect in the uptake and/or retention of norepinephrine exists in these hearts and that this defect may be responsible for the depletion of norepinephrine.
References


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