Reduction of Cardiac Tyrosine Hydroxylase Activity in Experimental Congestive Heart Failure

ITS ROLE IN THE DEPLETION OF CARDIAC NOREPINEPHRINE STORES

By Peter E. Pool, M.D., James W. Covell, M.D., Morton Levitt, Ph.D., James Gibb, Ph.D., and Eugene Braunwald, M.D.

Abstract

Although it is clear that cardiac norepinephrine stores are often markedly reduced in congestive heart failure, the mechanism responsible for this depletion has not been elucidated. The objective of this study was to investigate cardiac synthesis of norepinephrine in experimental right-sided heart failure by measuring the activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of norepinephrine. In homogenates of the right ventricles of 6 dogs with congestive heart failure and 2 with chronic cardiac denervation, myocardial tyrosine hydroxylase activity was severely reduced, averaging 0.4 ± 0.1 (SE) and 0.2 μmole/g per hour respectively as compared to a normal value of 3.3 ± 0.7 μmole/g per hour. Tyrosine hydroxylase activity was normal in reserpine-treated, norepinephrine-depleted dogs. These data provide evidence for a mechanism severely limiting norepinephrine biosynthesis in congestive heart failure.

Additional Key Words: biosynthesis catecholamines reserpine denervation enzyme activity anesthetized dogs

Tricuspid insufficiency and pulmonic stenosis

Methods

Experimental Preparations

From the Cardiology Branch and Laboratory of Clinical Biochemistry, National Heart Institute, Bethesda, Maryland.

Accepted for publication January 25, 1967.

Circulation Research, Vol. XX, March 1967

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were produced in 6 dogs, weighing between 15 and 25 kg, using the technique described by Barger and associates (11) and subsequently employed by other investigators (3, 12). At the time of study, 2 to 8 weeks following operation, the animals were anesthetized with intravenous sodium pentobarbital (12 mg/kg). Following determination of right atrial pressures, the heart was rapidly excised and kept at 0°C. All fat and connective tissue including valves were dissected free and cut away and the septum and the free walls of the right and left ventricle weighed.

An aliquot of each of the fat-free, connective tissue-free cardiac chambers was homogenized in a Virtis homogenizer for 1 min in 4 volumes of 0.32 M sucrose. Following centrifugation at 20,000 x g for 10 min, the supernatant was frozen for subsequent assay.

Reserpine (0.1 mg/kg) was administered to 4 normal dogs on 2 successive days. On the day following reserpine pretreatment, the heart was excised and homogenized as described. The hearts of 2 additional dogs that had undergone regional cardiac denervation using the technique of Cooper et al. (13) were treated in a similar manner. Seven dogs served as a control group.

**ASSAY TECHNIQUES**

The following materials were used: p-bromo-m-hydroxybenzoxamine (NSD 1055) was obtained from Smith Nephews, Ltd.; 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine (DMPH 4) from Aldrich Chemical Company; and L-tyrosine-U-14C (300 μc/μmole) from New England Nuclear Corporation. Interfering substances in the radioactive tyrosine were removed by treatment with alumina (14).

Tyrosine hydroxylase activity was measured by the conversion of tyrosine-14C to β-(3,4-dihydroxyphenyl)-L-alanine-14C (dopa) by a modification of the procedure described by Nagatsu et al. (15). The incubation mixture consisted of 0.04 μmole L-tyrosine (1.3 x 10^6 CPM), 400 μmole mercaptoethanol, 400 μmole phosphate buffer pH 6.5, 4 μmole DMPH 4, 4 μmole NSD 1055 and 3.6 ml of tissue extract in a final volume of 4.0 ml. The mixture was incubated at 37°C in air for 20 min on a metabolic shaker. The incubation was stopped by the addition of 7 ml of 10% trichloroacetic acid, and 20 μg of dopa was added as carrier. The radioactive dopa was isolated by alumina absorption as described by Crout (14). An aliquot of the alumina eluate was counted in Bray’s solution in a scintillation spectrometer. To express accurately the specific activity of tyrosine-14C, tissue tyrosine concentrations were determined by the fluorometric procedure of Waalkes and Udenfriend (16).

The tissue norepinephrine concentrations were assayed by the trihydroxyindole method (17, 18) after alumina absorption (19). Readings were made at activation wave lengths of 390 and 425 μm, and at fluorescence wave lengths of 500 and 525 μm, which allowed for the differentiation of norepinephrine and epinephrine.

**Results**

Mean right atrial pressure in the dogs with right-sided heart failure and chronic congestion averaged 19 cm H2O (range, 12 to 22 cm H2O). All animals had ascites (1.8 to 6.6 liter, average = 4.2 liter) and demonstrated gross pathologic evidence of chronic hepatic congestion. The ratio of right ventricular to left ventricular weight, excluding the septum, was significantly elevated in these dogs, averaging 0.86 ± 0.16 (se) compared to 0.48 ± 0.03 in control dogs (P < .05).

Tyrosine hydroxylase activity in the right ventricle and right atrium of the dogs with right-sided congestive heart failure was reduced (P < .01, Table 1). The tyrosine hydroxylase activity in the right ventricle was reduced from 3.3 ± 0.7 μmole/g per hour in the controls to 0.4 ± 0.1 μmole/g per hour in the animals in heart failure. In the right atria this reduction was from 4.9 ± 1.0 in normal animals to 0.5 ± 0.2 μmole/g per hour in the dogs with heart failure. Left atrial tyrosine hydroxylase activity was reduced from an average of 4.7 to 2.6 μmole/g per hour, a difference which was not statistically significant. Left ventricular tyrosine hydroxylase activity was not reduced. Tyrosine hydroxylase activity was also expressed as total activity of each chamber per kilogram body weight to insure that the reduction of tyrosine hydroxylase activity in the right atrium and ventricle of animals with heart failure was not due to dilution by an increased muscle mass. Expressed in this manner, tyrosine hydroxylase activity was reduced from an average of 4.0 ± 1.0 μmole/hr per kg body weight in the right ventricles of controls to 0.5 ± 0.2 (P < .01) in the ventricles of dogs with congestive heart failure. In the right atria, this reduction was from 1.0 ± 0.6 to 0.1 ± 0.1 μmole/hr per kg (P < .01). The average concentration of tyrosine in the right ven-
NOREPINEPHRINE SYNTHESIS IN HEART FAILURE

TABLE 1

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Tyrosine hydroxylase activity (mumole/g per hour) and norepinephrine concentration (ug/g) in homogenates of hearts from dogs with congestive heart failure (15.1 ± 1.3 ug/g) was similar to that in control hearts (13.8 ± 0.6 ug/g). In the 2 animals in which cardiac denervation had been carried out, right ventricular tyrosine hydroxylase activity was severely reduced to 0.2 and 0.3 mumole/g per hour in the right and left ventricles respectively. In contrast, reserpine pretreatment did not significantly alter tyrosine hydroxylase activity in any of the 4 cardiac chambers (Table 1).

Norepinephrine concentrations in the right ventricle and right atrium were significantly reduced in the failing hearts, averaging 0.09 ± 0.06 and 0.16 ± 0.06 ug/g respectively compared to 0.76 ± 0.14 and 1.66 ± 0.17 ug/g in the normal animals (P < .001, Table 1). The total right ventricular content of norepinephrine was also reduced, averaging 0.12 ± 0.09 ug/kg body weight compared to 0.84 ± 0.10 ug/kg body weight in control dogs. Norepinephrine concentrations in the left ventricle and left atrium were reduced to a lesser degree (Table 1). In the 4 reserpine-treated animals and in the 2 animals with cardiac denervation, norepinephrine stores also were markedly reduced (Table 1).

Discussion

The results of the present study demonstrate that tyrosine hydroxylase activity in homogenates of hearts from dogs with con-
gestive heart failure is markedly reduced. Since tyrosine hydroxylase has been shown to be the rate-limiting step in norepinephrine biosynthesis (10), this finding suggests that the depression of cardiac norepinephrine stores previously observed in congestive heart failure (1-4) may be related to a reduction in norepinephrine synthesis. Indeed, a highly significant correlation was evident when the norepinephrine concentration and tyrosine hydroxylase activity were related to one another in the individual chambers of both normal and failing hearts (Fig. 1).

Cardiac denervation was also accompanied by a reduction in tyrosine hydroxylase activity. Since this enzyme is probably localized to the sympathetic nerve endings (9), this depression in enzymatic activity is compatible with the reduction in the number of sympathetic nerve endings which occurs in denervated hearts (20, 21) and is additional evidence for the localization of norepinephrine in the sympathetic nerves. On the other hand, reserpine, which profoundly depletes norepinephrine stores by accelerating the release of norepinephrine from its storage site (22), does not significantly affect tyrosine hydroxylase activity (23).

Previously it has been shown that the cardiac uptake of norepinephrine in experimental heart failure may be reduced to approximately the same extent as is the norepinephrine concentration and that the turnover of norepinephrine in the failing heart is normal relative to the remaining neurotransmitter stores (4). In the present study evidence has been presented for a reduction in norepinephrine biosynthesis that is also proportional to the reduction in norepinephrine concentration (Fig. 1). Therefore, the available data when taken together support the hypothesis that in congestive heart failure there is a parallel reduction in the synthesis, uptake and binding of norepinephrine, rather than a specific disturbance of one of these functions.

*Circulation Research, Vol. XX, March 1967*
Acknowledgment

The authors gratefully acknowledge the advice of Drs. S. Udenfriend and C. A. Chidsey, and the technical assistance of Shirley C. Seagren.

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Circ Res. 1967;20:349-353
doi: 10.1161/01.RES.20.3.349

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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