CARDIAC SYMPATHETIC INNERVATION IN PATIENTS WITH DILATED CARDIOMYOPATHY

— Immunohistochemical Study Using Anti-Tyrosine Hydroxylase Antibody —

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Depletion of norepinephrine in the left ventricular myocardium in cases of dilated cardiomyopathy (DCM) has been suggested. However, there have been few histological studies of the sympathetic nerves, in which myocardial norepinephrine is believed to exist. We performed an immunohistological study of the density of tyrosine hydroxylase (TH, a marker of sympathetic nerves)-positive nerve fiber in endomyocardial biopsy specimens in cases of DCM using antibody against TH. TH-positive nerves were stained brown along with the myocardium, and they were more dense in the right ventricle than in the left ventricle in both the DCM and control groups. The density of TH-positive nerves in cases of DCM was significantly less than that in the control group in the subendocardial myocardium of the right and left ventricles, but especially in the left ventricle. A correlation was observed in the DCM group between the density of TH-positive nerves and the ejection fraction in the right ventricle, but not in the left ventricle. In the failing human heart, a decrease in subendocardial sympathetic nerve density may be one of the causes of myocardial norepinephrine depletion. (*Jpn Circ J* 1994; **58**: 389–394)

ELEVATION of the serum norepinephrine concentration with a decrease in the myocardial norepinephrine concentration has been reported in patients with dilated cardiomyopathy (DCM)^{1–4} Moreover, some studies using ¹²³I-metaiodobenzylguanidine-myocardial scintigraphy have indicated decreased reuptake, lack of uniformity, and accelerated washout in DCM patients.^{5–7} The metabolism of norepinephrine in sympathetic nerve endings is shown in Fig. 1.^{8,9} The activity of norepinephrine released from vesicles by sympathetic stimulation develops due to binding with β -receptor in the myo-

cardial cellular membrane; some norepinephrine (70–80%) is taken up in the vesicles, and the remainder flows into the blood.

The activity of each sympathetic nerve is reportedly accelerated in patients with DCM.¹⁰ Moreover, myocardial norepinephrine is believed to exist primarily in pre-synaptic storage vesicles in the sympathetic terminals. Therefore, to explain a decrease in myocardial norepinephrine, one must consider changes in sympathetic nerve density and depletion of norepinephrine storage vesicles. However, there have been few histological studies of the distribution of myocardial sympathetic nerves, and this point remains to be elucidated. Thus, in the present study, we studied the density of sym-

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Fig.1. Metabolism of norepinephrine in sympathetic nerve endings. Tyrosine incorporated in the sympathetic terminal is converted to dihydroxyphenylalanine (DOPA) by TH, synthesized to norepinephrine via dopamine, and then stored in presynaptic storage vesicles in the sympathetic terminal.

pathetic nerves immunohistochemically using TH¹¹⁻¹⁶ as a marker of sympathetic nerve terminals in patients with DCM.

MATERIALS AND METHODS

Biopsy specimens were obtained during routine diagnostic cardiac catheterization from the right and left ventricles in 13 patients with DCM in the Third Department of Internal Medicine of Showa University Hospital, from November 1991 to October 1992. The patients ranged in age from 31 to 71 years (12 males, 1 female; mean age, 55.4 years). The diagnosis of DCM was made according to the definition and classification proposed by the World Health Organization/International Society and Federation of Cardiology Task Force¹⁷ Table I shows the clinical profiles and hemodynamics of the DCM patients. 9 patients (6 males, 3 females; age, 41 to 72 years; mean, 58.6 years) with normal pump function and coronary arteries served as the control group, even though angina pectoris was suspected. We explained the necessity, safety and risk of endomyocardial biopsy to all the patients, and obtained their consent.

Biopsy specimens obtained from both ven-

TABLE I

Case	Age (yr)	Sex	EF (%)	CI (l/min/m ²)	PCWP (mmHg)
1	52	M	30.6	2.25	5.2
2	41	M	43.2	2.27	9.9
3	63	M	18.3	2.50	10.4
4	64	M	17.0	2.07	8.2
5	70	M	26.7	2.31	7.2
6	71	M	37.4	2.78	9.2
7	67	M	25.5	2.60	8.4
8	64	M	15.0	1.99	18.2
9	44	M	29.1	2.36	4.2
10	59	M	27.9	4.17	8.0
11	31	M	35.7	3.73	6.8
12	49	F	29.5	2.49	25.0
13	45	M	29.7	2.13	10.5
Mean	55.4		28.12	2.59	10.08
SD	12.1		7.76	0.62	5.39

EF: ejection fraction; CI: cardiac index;
PCWP: pulmonary capillary wedge pressure.

tricles at cardiac catheterization were immediately fixed with Zamboni's solution for 90 min, and specimens were washed out overnight with 20% sucrose-added phosphate buffered saline. After these specimens were embedded at optimal cutting temperature (OCT) in embedding compound (Miles Laboratories, Inc.), they were rapidly frozen by n-hexane at -80°C , and 10 μm -thick frozen sections were prepared at -20°C . Immunohistochemical staining was carried out by the labeled streptavidin biotin (LSAB) method using anti-TH antibody. Adrenal glands of rats served as positive controls. Each specimen was then observed in 5 high-power visual fields, and the number of positive nerve fibers per visual field (0.2 mm²) was compared with that in the control group. Specimens in which the myocardial cells had been replaced by fibrous tissue were excluded. Comparisons were made by the Wilcoxon test.

RESULTS

TH-positive nerves in the left ventricle in the control and DCM groups are shown in the photomicrographs in Fig. 2 and 3, respectively. Positive nerves that were stained brown along with the myocardium in the DCM and control groups are indicated by

Fig.2. TH-positive nerves in a left ventricle in the control group (indicated by arrows). Positive nerves were stained brown along with myocardium.

Fig.3. TH-positive nerve in a left ventricle in the DCM group (indicated by arrow). The density of TH-positive nerves in the DCM group was significantly less than that in the control group.

arrows.

The number of TH-positive nerves in the right ventricle in the DCM group (4.95 ± 4.89) was significantly less than that in the

control group (12.31 ± 3.41 ; approximately 60% less) ($p < 0.05$). Similarly, the number of TH-positive nerves in the left ventricle in the DCM group (2.78 ± 2.59) was also signif-

Fig.4. The number of TH-positive nerves per visual field (0.2 mm^2) in both ventricles in the DCM group was significantly less than that in the control group. In the control group, TH-positive nerves were observed significantly more often in the right ventricle than in the left ventricle. Although a similar pattern was observed in the DCM group, the difference here was not significant.

Fig.5. A correlation was found between the number of TH-positive nerves and ejection fraction (%) in the right ventricle of the DCM group.

icantly less than that in the control group (9.71 ± 1.59 ; approximately 70% less) ($p < 0.01$). The density of sympathetic fibers was also significantly less in the DCM group. In the control group, more TH-positive nerves were observed in the right ventricle than in

Fig.6. No correlation was observed between the number of TH-positive nerves and ejection fraction (%) in the left ventricle of the DCM group.

the left ventricle ($p < 0.05$). Although a similar pattern was observed in the DCM group, the difference here was not statistically significant (Fig. 4). In the DCM group, a correlation was observed between the number of TH-positive nerves and the ejection fraction in the right ventricle (Fig. 5), but not in the left ventricle (Fig. 6). No correlation was found between the number of TH-positive nerves and cardiac index or pulmonary capillary wedge pressure in either ventricle. The ejection fraction in the DCM group (28.12 ± 7.76) was significantly less than that in the control group (63.91 ± 7.37) ($p < 0.01$). There was no statistically significant difference in either the cardiac index or the pulmonary capillary wedge pressure between the DCM group (2.59 ± 0.62 and 10.08 ± 5.39 , respectively) and the controls (3.18 ± 0.86 and 9.33 ± 3.47 , respectively).

DISCUSSION

The present results suggest that DCM patients have a reduced subendocardial sympathetic nerve density. However, it is not clear whether this reduced myocardial sympathetic nerve density in DCM patients is a factor in producing heart failure or whether it is a response to the enhanced norepinephrine blood level due to heart failure dynamics. Cohn and Levine reported a correlation between blood norepinephrine levels and the prognosis of patients with

heart failure¹⁸ However, Heilbrunn and Shah reported that myocardial cellular disturbance may occur due to a state of continuous tension in the sympathetic nerves!¹⁹ Furthermore, Leimbach reported that sympathetic nerve activity is accelerated in DCM patients!¹⁰

TH-positive nerves were more dense in the right ventricle than in the left ventricle in both the control and DCM groups. Shvalev and Stropus reported similar results in a study of hearts obtained from autopsy cases!²⁰ Moreover, this density in both the left and right ventricles was significantly less in the DCM group than in the control group. However, a correlation between density and ejection fraction, which is generally considered to reflect the prognosis,²¹⁻²³ was observed only in the right ventricle. This suggests that the decrease in myocardial sympathetic nerve density is a result, rather than a cause, of heart failure, since there is a correlation between right ventricular findings and progression of this disease, even when DCM is originally a left cardiac lesion.

Pierpont and Francis reported that the myocardial catecholamine concentration in recipients with heart failure at cardiac transplantation does not vary with advancement of the disease, and also observed remarkable local differences in each part of the myocardium!²⁴ In the present study, we believed that sampling error was likely since the left ventricle was thicker than the right ventricle.

In this study, we also examined the number of nerves per unit area to calculate the number of sympathetic nerves. It is a possible that the number of nerves should be corrected by the number of myocardial cells or by the myocardium/stroma ratio in the unit area, since some DCM patients showed significant myocardial hypertrophy. However, we did not make such corrections because we could not align the myocardial fibers in all of the thin sections in the same direction due to the biopsy specimens that were available.

Sympathetic innervation may take place in either the epicardium or the endocardium, since sympathetic nerves are distributed from the epicardium to the endocardium. This sympathetic innervation may require further autopsy investigation. However, the antibody used in the present study precluded

immunohistochemical staining after a long post-mortem interval. Therefore, we used only endomyocardial biopsy specimens.

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