DUAL-TRACER AUTORADIOGRAPHY WITH THALLIUM-201 AND IODINE-125 MIBG IN BIO 14.6 CARDIOMYOPATHIC SYRIAN HAMSTERS

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Dual-tracer imaging of the heart with ^{125}I-metaiodobenzylguanicline (MIBG) and ^{201}Tl can simultaneously demonstrate the distribution of sympathetic nerve endings and the underlying myocardial perfusion. A quantitative dual-tracer autoradiographic study with ^{201}Tl and ^{125}I-MIBG was performed to investigate changes in the distribution of cardiac sympathetic innervation with the progression of cardiomyopathy in BIO 14.6 hamsters. The distribution of ^{201}Tl was uniform in control hamsters and BIO 14.6 hamsters at all stages of cardiomyopathy. In contrast, a reduction in MIBG accumulation occurred in the endocardial region of the left ventricular free wall and the left ventricular aspect of the interventricular septum in BIO 14.6 hamsters at 3 and 8 months of age. Thus, there was an uncoupling of the left ventricular distribution of ^{201}Tl and ^{125}I-MIBG in BIO 14.6 hamsters. In addition, interstitial fibrosis was increased in the interventricular septum, the subendocardial region of the left ventricular free wall, and the right ventricular wall, which were the sites of reduced MIBG accumulation. This study shows that dual myocardial imaging with MIBG and ^{201}Tl may be useful for investigating patients with cardiomyopathy.

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THERE is considerable evidence that sympathetic nervous dysfunction occurs in the hearts of humans with congestive heart failure[1] and in animals with experimentally induced heart disease[2,3]

The BIO 14.6 cardiomyopathic Syrian hamster is an animal model of human idiopathic cardiomyopathy, and generally develops myocardial necrosis, fibrosis, and calcification 30 days after birth, followed by cardiac hypertrophy at 90–120 days and congestive heart failure at 160–200 days[4] It has been suggested that abnormalities of the

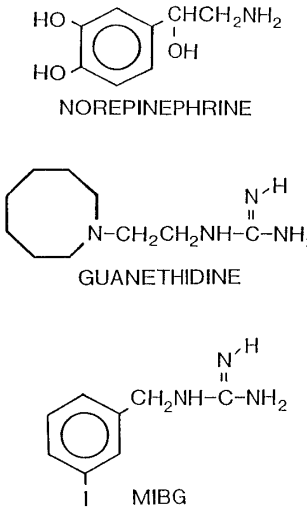

Fig.1. Chemical structures of norepinephrine, guanethidine, and metaiodobenzylguanidine (MIBG).

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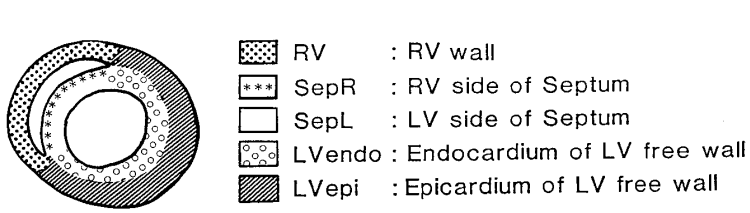

Fig.2. Each heart section was divided into five regions, and each region was digitized and quantitated using a videodensitometry system.

sympathetic nervous system may contribute to the pathogenesis and development of cardiac hypertrophy and heart failure in this animal[5,6] Sole et al[7] reported that the myocardial norepinephrine content was significantly increased in the necrotic and hypertrophic stages of cardiomyopathy in this animal, but then declined during the late stage of hypertrophy and heart failure. Angelakos et al[8] examined noradrenergic nerve endings in BIO 14.6 hamster hearts using formaldehyde fluorescence histochemistry, and demonstrated an increase in the fluorescence intensity of the nerve terminals. However, they did not investigate the distribution of myocardial noradrenergic nerve endings. Kawai et al[9] showed that the myocardial norepinephrine content was lower in the left ventricle than in the right ventricle of patients with dilated or hypertrophic cardiomyopathy, indicating that the distribution of catecholamines in the heart is not uniform.

Metaiodobenzylguanidine (MIBG) is an analogue of guanethidine (Fig. 1) that shows affinity for the adrenal medulla and sympathetic nerve endings, including those of the heart[10] and which is believed to have uptake and storage mechanisms similar to those of norepinephrine[11] Thus, MIBG provides a useful tool for studying changes in sympathetic innervation.

We performed a quantitative dual tracer autoradiographic study using ^{201}Tl (which is believed to reflect changes in coronary perfusion and histology) and ^{125}I-MIBG (reflecting sympathetic innervation) to investigate changes of myocardial sympathetic innervation during the progression of cardiomyopathy in the BIO 14.6 cardiomyopathic hamster.

MATERIALS AND METHODS

Animals

BIO 14.6 cardiomyopathic Syrian hamsters were obtained from BIO Breeders Inc. (USA) and maintained at the Institute for Experimental Animals at Hamamatsu University School of Medicine. All experiments conformed to the Hamamatsu University School of Medicine regulations governing the care and use of laboratory animals. Sixteen BIO 14.6 hamsters were divided into the following three age groups; 4 animals aged 1 month (early necrotic stage of cardiomyopathy), 8 animals aged 3 months (hypertrophic stage), and 4 animals aged 8 months (heart failure stage). Eighteen age-matched normal F1b hamsters were used as controls and divided into the same three age groups.

Quantitative Autoradiography

All animals were first injected intravenously with ^{125}I-MIBG (1.85 mBq), and were then administered ^{201}Tl (11.84–23.68 MBq) 230 min later. Hearts were removed 10 min after the injection of ^{201}Tl, frozen in liquid nitrogen, and embedded in carboxylmethyl cellulose. The hearts were sectioned perpendicular to the longitudinal axis of the left ventricle using a cryomicrotome. Myocardial sections 20 μm thick were placed on X-ray films and the first autoradiographic exposure was carried out for 11 h to determine the distribution of ^{201}Tl. The second exposure was begun 30 days later following the decay of ^{201}Tl activity, and autoradiography of ^{125}I-MIBG required 50 days to obtain an adequate image quality. In a preliminary study involving single-tracer autoradiography using each tracer under the same conditons, it was confirmed that neither ^{201}Tl nor ^{125}I-MIBG were visualized under the conditions used for imaging the other tracer.

To quantitate the myocardial distribution of ^{201}Tl and ^{125}I-MIBG, each heart section was divided into the following five regions:

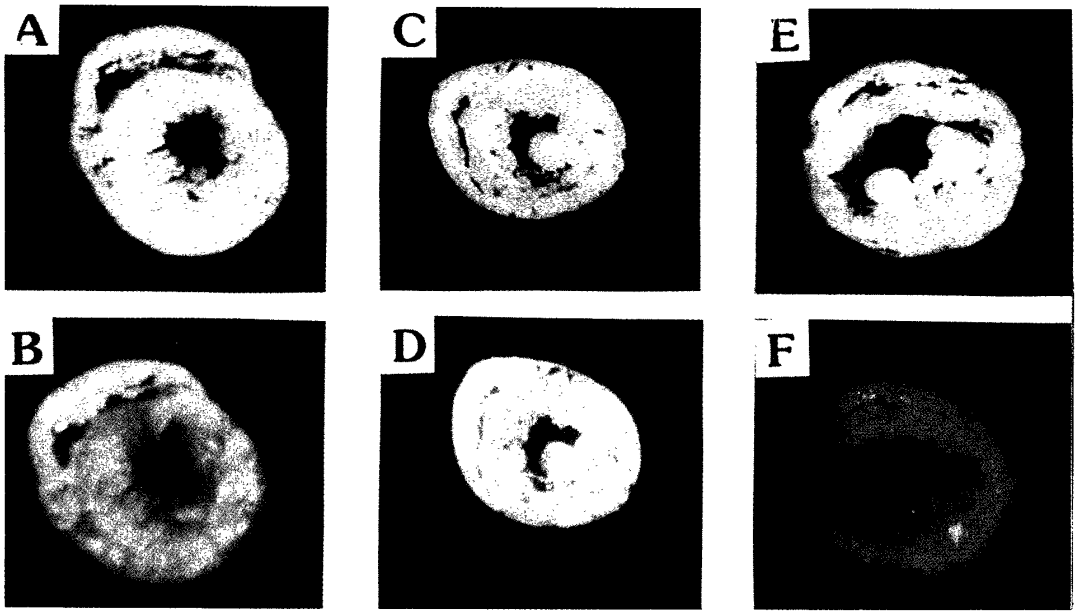

Fig.3. Representative digitized dual tracer autoradiograms with ^{201}Tl and ^{125}I-MIBG in F1b and BIO 14.6 hamsters. A ^{201}Tl autoradiogram of a 3-month-old F1b hamster shows a uniform distribution (A). A ^{125}I-MIBG autoradiogram of the same section as (A) shows a high uptake in the right ventricular wall (B). A ^{201}Tl autoradiogram of a 3-month-old BIO 14.6 hamster shows a uniform distribution except for diffusely scattered small defects (C). A ^{125}I-MIBG autoradiogram of the same section as (C) shows a high uptake in the right ventricular wall (D). In an 8-month-old hamster, a ^{125}I-MIBG autoradiogram (F) shows a heterogeneous distribution, which is markedly different from that of ^{201}Tl (E).

the right ventricular wall, the right ventricular aspect of the interventricular septum, the left ventricular aspect of the interventricular septum, the endocardial region of the left ventricular free wall, and the epicardial region of the left ventricular free wall (Fig. 2). Each selected region of the ^{201}Tl and ^{125}I-MIBG autoradiograms was digitized and quantitated using a videodensitometry system and a CCD camera (Sony, Japan) connected to an image processor(TOSPIX, Toshiba, Japan).

The relative uptake of each tracer was defined as the ratio of the regional optical density to the maximum optical density in the five regions. This relative uptake reflects the relative distribution of tracer in the myocardium. Fig. 3 shows examples of ^{201}Tl and ^{125}I-MIBG images obtained in BIO 14.6 hamsters and control F1b hamsters.

Biodistribution Study

^{125}I-MIBG alone was injected intravenously into 6 control hamsters aged 8 months and 6 BIO 14.6 hamsters aged 8 months to evaluate myocardial uptake. Four hours after the injection, hearts were removed, weighed and counted in an autogamma counter. To normalize for differences in animal weights, tissue concentrations were expressed as percentage kilogram dose per gram (%kg dose/g).

Histological Study

Myocardial sections (5 μm) were employed for a histological study of 8-month-old BIO 14.6 hamsters. The sections were stained with haematoxylin and eosin, Masson's trichrome, and von Kossa's stain.

Statistical Analysis

All relative uptake data are expressed as the mean$\pm$SD. Student's t test was used for comparing the results from two groups. One-way analysis of variance was used for comparisons among the five regions. Where a significant difference was obtained among the five regions, the Duncan test was used for multiple comparisons. A p value <0.05 was considered to indicate statistical significance.

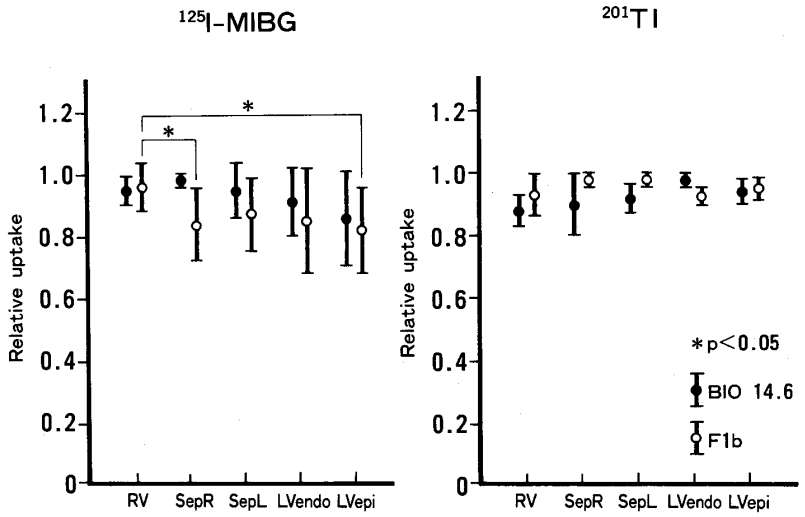

Fig.4. Relative uptake of ^{125}I-MIBG and ^{201}Tl in 1-month-old hamsters. All values are the mean±SD. Open circles indicate control F1b hamsters (N=6) and closed circles indicate BIO 14.6 hamsters (N=4). Although the relative uptake of ^{125}I-MIBG varied significantly among the five regions in the control hamsters ($p<0.05$), it was similar in the BIO 14.6 hamsters ($p<0.05$). On the other hand, the relative uptake of ^{201}Tl in the five regions was similar in both the control and BIO 14.6 hamsters ($p>0.05$). RV; right ventricular wall, SepR; right ventricular aspect of the interventricular septum, LVendo; endocardial region of the left ventricular free wall, LVepi; epicardial region of the left ventricular free wall.

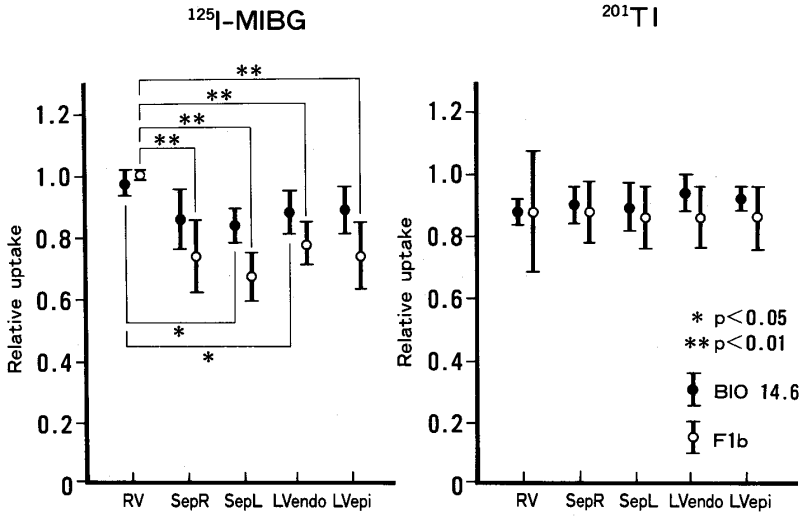

Fig.5. Relative uptake of ^{125}I-MIBG and ^{201}Tl in 3-month-old hamsters. All values are the mean±SD. In both the control and BIO 14.6 hamsters (N=6 and N=8, respectively), the relative uptake of ^{125}I-MIBG was significantly different among the five regions ($p<0.01$ and $p<0.05$, respectively), although that of ^{201}Tl was similar among the five regions (both $p>0.05$).

RESULTS

Dual-tracer Autoradiography in Control F1b Hamsters

The relative uptakes of ^{201}Tl and ^{125}I-MIBG in 1-, 3, and 8-month-old control F1b hamsters are shown as open circles in Fig. 4, 5 and 6, respectively. In the control hamsters, left ventricular imaging was uniform with both ^{201}Tl and ^{125}I-MIBG. The relative uptake of ^{201}Tl by the right ventricle did not significantly differ from that in any of the left ventricular regions, but the relative uptake of ^{125}I-MIBG was significantly higher in the right ventricle than in any of the left ventricular regions.

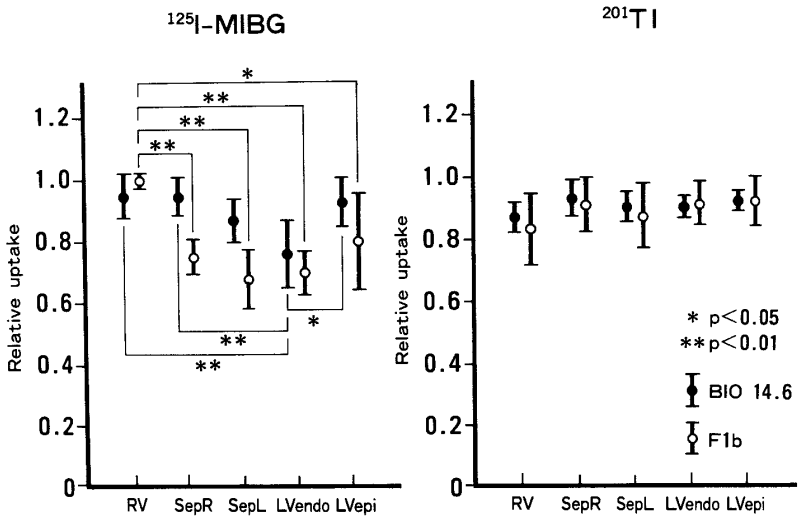

Fig.6. Relative uptake of ^{125}I-MIBG and ^{201}Tl in 8-month-old hamsters. All values are the mean±SD. In both the control and BIO 14.6 hamsters (N=6 and N=4, respectively), the relative uptake of ^{125}I-MIBG was significantly different among the five regions (both $p<0.01$), although that of ^{201}Tl was similar among the five regions (both $p>0.05$).

Dual-tracer Autoradiography in BIO 14.6 Hamsters

The relative uptakes of ^{201}Tl and ^{125}I-MIBG in 1-, 3, and 8-month-old BIO 14.6 hamsters are shown as closed circles in Fig. 4, 5 and 6, respectively. The relative uptake of ^{201}Tl was not significantly different among the five heart regions in the BIO 14.6 hamsters, as was the case in the control F1b hamsters. The relative uptake of ^{125}I-MIBG did not significantly differ among the five regions in 1-month-old BIO 14.6 hamsters (Fig. 4). However, this uptake was not uniform in 3- and 8-month-old BIO 14.6 hamsters (Fig. 5 and 6: $p<0.05$ and $p<0.01$, respectively). In the 3-month-old BIO 14.6 hamsters, the relative uptake of ^{125}I-MIBG was significantly lower in the left ventricular aspect of the interventricular septum and endocardial region of the left ventricular wall than in the right ventricular wall (Fig. 5: $p<0.05$). In the 8-month-old BIO 14.6 hamsters, the relative uptake of ^{125}I-MIBG was significantly lower in the endocardial region of the left ventricular wall than in the right ventricular wall, the right ventricular aspect of the septum, and epicardial region of the left ventricular wall (Fig. 6: $p<0.01$, $p<0.01$ and $p<0.05$, respectively).

Biodistribution Study

Cardiac uptake in control hamsters was not significantly different from that in cardiomyopathic hamsters (0.36±0.04 versus 0.38±0.15 %kg dose/g, $p>0.05$)

Histological Study

The areas of myocardial necrosis and calcification in the 8-month-old BIO 14.6 hamsters were small and widely scattered (Fig. 7). Interstitial fibrosis was mildly increased in the interventricular septum, the subendocardial region of the left ventricular free wall, and the right ventricular wall.

DISCUSSION

The BIO 14.6 cardiomyopathic hamster develops a hereditary cardiomyopathy that terminates in congestive heart failure[4] During the first month of life, BIO 14.6 hamsters appear to be healthy and there is no pathological evidence of heart disease. However, focal myocardial necrosis appears after 1 month of age. By 3 to 4 months of age, many of these necrotic lesions have healed and few new lesions appear. Subsequently, cardiac hypertrophy begins to develop and calcification of the degenerating muscle occurs. After 6 months of age, cardiac dilation develops, and many animals die from congestive heart failure by 1 year of age.

There was no marked difference in the ^{201}Tl autoradiograms of control F1b and BIO 14.6 hamsters at any stage of cardiomyopathy. Although very small defects were ob-

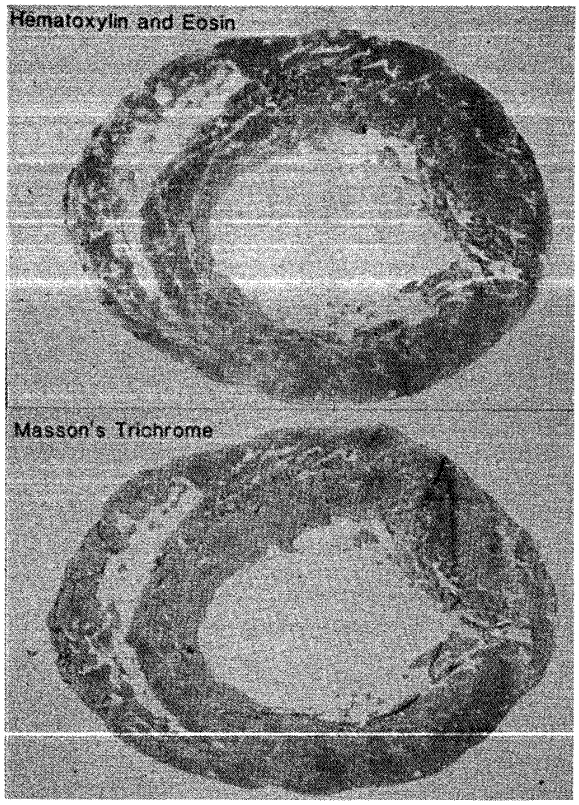

Fig.7. Histological appearance of the heart in an 8-month-old BIO 14.6 hamster. The sections were stained with haemotoxylin and eosin (A) and Masson's trichrome (B).

served in the BIO 14.6 hamsters at 8 months of age, which were thought to reflect histologic changes such as necrosis, fibrosis, and calcification, these defects were too small and too diffusely scattered to be detected by the relative regional tracer uptake analysis that was performed in this study. Thus, there were no marked abnormalities of myocardial ^{201}Tl distribution in the BIO 14.6 hamsters during the progression of cardiomyopathy, at least up to the end of our study.

MIBG is thought to have uptake and storage mechanisms similar to those of norepinephrine;[10] and competition between the uptake of MIBG and circulating catecholamines occurs in the heart![11] The distribution of MIBG most closely represents the distribution of sympathetic nerve endings with a normal uptake mechanism![12] Therefore, imaging with MIBG is a useful tool for studying changes in sympathetic innervation![13]

In the control F1b hamsters, the right ventricle had a higher relative uptake of ^{125}I-MIBG than any region of the left ventricle. Whether increased innervation of the right ventricle is of any physiologic significance remains unknown at present, although a higher norepinephrine concentration in the right ventricle than in the left ventricle has also been found in the rat, guinea pig, rabbit, and cat?[3,14] On the other hand, ^{125}I-MIBG uptake was uniform in all regions of the right and left ventricles in 1-month-old BIO 14.6 hamsters. Since the myocardial norepinephrine content is significantly increased during the early necrotic and hypertrophic stages of BIO 14.6 cardiomyopathic hamsters[8] the homogeneous nature of MIBG uptake in the 1-month-old hamsters may be mediated by increased uptake in the left ventricle rather than by decreased uptake in the right ventricle.

^{125}I-MIBG uptake was decreased in the left ventricular aspect of the interventricular septum in BIO 14.6 hamsters at 3 months of age, and also in the endocardial region of the left ventricular free wall at 3 and 8 months of age. Thus, the older BIO 14.6 hamsters had more distinct regional heterogeneity of left ventricular distribution of ^{125}I-MIBG. In contrast, ^{201}Tl uptake at any given age was uniform in all regions of the heart. Thus, there was an uncoupling of the left ventricular ^{201}Tl and ^{125}I-MIBG distribution in BIO 14.6 hamsters at 3 to 8 months of age. The regional uptake of ^{125}I-MIBG changed independently of regional myocardial perfusion, because ^{201}Tl uptake remained uniform. In addition, since the biodistribution study showed that cardiac uptake was similar in 8-month-old control and cardiomyopathic hamsters, the regional heterogeneity may be a more important alteration than total changes in sympathetic innervation of the myocardium.

The possible mechanisms which underlie the heterogenous left ventricular distribution of ^{125}I-MIBG in 3- and 8-month-old BIO 14.6 hamsters may be explained as follows. MIBG is more firmly bound inside storage vesicles than outside the vesicle![15] In 3-month-old BIO 14.6 hamsters, cardiac norepinephrine turnover is increased, suggesting that there is an increase of adrenergic sympathetic nervous activity![8] If cardiac norepinephrine turnover is maximal in the septal region, norepinephrine could efflux from intraneuronal to extravesicular sites, and

would then undergo faster efflux from the myocardium. This mechanism provides one possible explanation for the heterogenous distribution of ^{125}I-MIBG in the left ventricular aspect of the interventricular septum in 3-month-old BIO 14.6 hamsters.

Kawai et al reported that the norepinephrine content is higher in the right ventricle than in the left ventricle in patients with congestive cardiomyopathy, and suggested that the decrease observed in the left ventricle may be due to simple dilution of sympathetic nerve endings by the hypertrophic left ventricular hypertrophic myocardial cells[9] If the dilution of sympathetic nerve endings actually caused the reduction of ^{125}I-MIBG accumulation, the distribution of this tracer would be homogeneously decreased in all regions of the left ventricle. However, since the relative uptake of ^{125}I-MIBG was only decreased in the left ventricular aspect of the interventricular septum and the endocardial region of the left ventricular free wall, this change cannot be explained by simple dilution of sympathetic nerve endings due to cardiac hypertrophy.

Eight-month-old BIO 14.6 hamsters have congestive heart failure. There is considerable evidence of a decrease and regional heterogeneity of myocardial catecholamines in patients and animals with congestive heart failure[9,16] In addition, Sole et al[7] have reported that the cardiac norepinephrine content declines during heart failure in BIO 14.6 hamsters. Amorim et al[17] have demonstrated that the heart tends to have a reduced number of sympathetic neurons in congestive cardiomyopathy. If there were fewer neurons to accumulate MIBG, then it could follow that a larger proportion of the tracer would remain in extraneuronal sites. Our histological study showed that interstitial fibrosis was increased in the interventricular septum, the subendocardial region of the left ventricular free wall, and the right ventricular wall in BIO 14.6 hamsters at 8 months of age. These were the same sites where MIBG accumulation was reduced. Thus, the number of sympathetic neurons may have been reduced in 8-month-old BIO 14.6 hamsters as a result of fibrosis, and this may have led to the decrease of ^{125}I-MIBG uptake in the right ventricular wall and the endocardial region of the left ventricular free wall.

We have already reported that the myocardial distribution of β-adrenergic receptors was heterogeneous in 280-day-old BIO 14.6 hamsters with an increase in the septal and subendocardial regions[18] Thus, the regions of increased interstitial fibrosis also corresponded to the sites of increased β-adrenergic receptor density. We suggest that the increases in β-adrenergic receptor density and norepinephrine turnover in the septal and subendocardial regions of the left ventricle may induce hypersensitivity to catecholamines, which may be followed by tissue damage and interstitial fibrosis, as well as by a reduction in the number of sympathetic neurons in the later stages of cardiomyopathy. Henderson et al[19] demonstrated that images obtained from cardiomyopathy patients showed a patchier distribution of MIBG activity than images from normal controls, and our results agree well with their findings.

In conclusion, functional ^{201}Tl and MIBG imaging can simultaneously demonstrate the distribution of sympathetic nerve endings and the underlying myocardial coronary perfusion. This method may be particularly useful for determining whether a structural or function imbalance of sympathetic nervous innervation is present in patient with cardiomyopathy.

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