Assessment of Adrenergic Neuron Function Altered with Progression of Heart Failure

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We used MIBG to evaluate cardiac adrenergic neuron integrity and function in congestive heart failure. **Methods:** Rats were treated with adriamycin (2 mg/kg, s.c.) once a week for 7, 8 and 9 wk. In analyzing cardiac adrenergic neuron function, we assessed alterations of uptake-1, exocytotic release and nonexocytotic metabolic release in relation to progression of heart failure. **Results:** LVEF progressively decreased. Cardiac MIBG accumulation (4 hr postinjection) decreased to 53% of control at 7 wk and markedly decreased to 14% of control at 9 wk, accompanied by massive pleural effusions. Reduction of MIBG accumulation in the lung and spleen, which are adrenergic-rich organs similar to the heart, were less pronounced compared to reduction in the heart. There was no difference in cardiac uptake of ^{3}H-norepinephrine between the control and 8-wk groups. Cardiac uptake of ^{3}H-norepinephrine decreased 91.0% in the control and 90.8% in the 8 wk group by pretreatment of desipramine, indicating no difference in the uptake-1 component. **Conclusion:** Congestive heart failure due to adriamycin cardiomyopathy progressively accelerates exocytotic release of norepinephrine predominantly from cardiac adrenergic neurons, but neuronal uptake function is not disturbed so long as heart failure is not advanced. In the advanced stage, nonexocytotic metabolic release is induced specifically in cardiac adrenergic neurons due to energy depletion and norepinephrine release markedly increases.

Key Words: congestive heart failure; adrenergic neuron function; metaiodobenzylguanidine; adriamycin

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Adrenergic nervous system activity is increased overall in congestive heart failure (*1–5*). Although it was previously suggested that there was a functional denervation of the heart in heart failure, based on findings of reduced myocardial norepinephrine stores (*6,7*) and reduced myocardial norepinephrine synthesis and turnover (*8,9*), more recent studies (*4,10–12*) have shown an increase in the rate of norepinephrine spillover to plasma from the heart, suggesting cardiac adrenergic hyperactivation. Controversy exists whether increased cardiac norepinephrine spillover could result from either increased adrenergic neuronal release of norepinephrine (*4,12*) or from impaired cardiac neuronal reuptake (uptake-1) (*13,14*).

Furthermore, increased neuronal release of norepinephrine may be induced by acceleration of exocytotic release due to both systemic and local cardiac adrenergic activation and also by nonexocytotic metabolic release which has been demonstrated under conditions of energy depletion in myocardial ischemia, anoxia or cyanide intoxication (*15*).

Recently, radiolabeled metaiodobenzylguanidine (MIBG), an analog of norepinephrine which serves as an index of adrenergic neuron integrity and function, was developed (*16,17*). MIBG may be more reliable than norepinephrine in its disappearance from the heart since MIBG is metabolized to only a small extent and does not bind to postsynaptic receptors (*17*). Myocardial accumulation of MIBG with a high specific radioactivity, determined 4 hr after intravenous injection can be regarded as a reflection of cardiac adrenergic neuron activity (*18*).

Adriamycin cardiomyopathy is a dose-dependent process which results in myocyte damage that culminates in congestive heart failure. In our previous studies (*18,19*) using a rat model with adriamycin cardiomyopathy, it was demonstrated that abnormal cardiac adrenergic neuron activity, as expressed by marked reduction of MIBG accumulation in the heart, showed a greater and more linear dose-dependent exacerbation than left ventricular ejection fraction (LVEF). Although decrease of myocardial norepinephrine concentration and increase of plasma norepinephrine levels in adriamycin treatment rats were significant, changes of these parameters were not dose-dependent. It is suggested that myocardial MIBG accumulation may be a more sensitive indicator for cardiac adrenergic activity.

Evaluation of cardiac adrenergic neuron integrity and function using radiolabeled MIBG may result in the interpretation of abnormal cardiac adrenergic neuron activity in congestive heart failure. Therefore, we assessed alterations of uptake-1, exocytotic release and nonexocytotic metabolic release in relation to progression of heart failure in a rat model with adriamycin cardiomyopathy.

MATERIALS AND METHODS

Adriamycin was supplied from Kyowa Hakko Kogyo Co., Ltd (Japan). Iodine-125-MIBG (specific activity: 100 mCi/mg) was purchased from Daiichi Radioisotope Laboratory (Tokyo, Japan).

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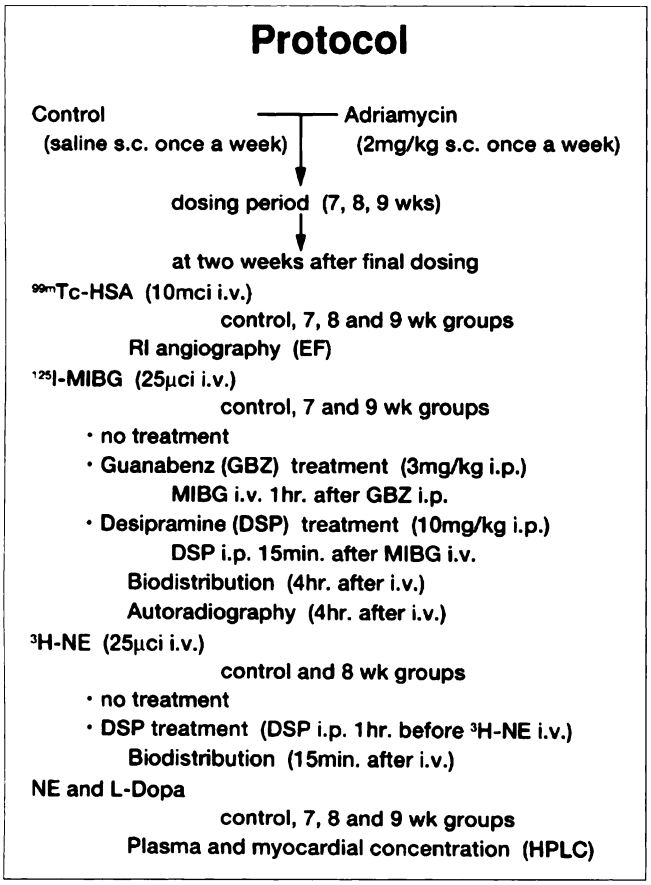

FIGURE 1. Study protocol.

Radiochemical purity was >99%, as determined by silica gel (ethyl acetate:ethanol (1:1), Rf = 0.30) and reversed phase TLC (80% methanol Rf = 0.05). Tritiated norepinephrine, levo-[ring-2,5,6-^{3}H] had a specific activity of 48.6 Ci/mmol and a radiochemical purity >99%.

Male Wistar rats, weighing 250 ± 20 g were treated with adriamycin (2 mg/kg, s.c.) once a week for 7, 8 and 9 wk (Fig. 1). Control rats were injected with saline. At 2 wk after the final adriamycin treatment in each group, radionuclide angiography with ^{99m}Tc-HSA (10 mCi) was performed in the control and 7-, 8- and 9-wk groups. LVEF was calculated from gated blood-pool images as previously described (*19*). Iodine-125-MIBG (25 μCi/kg) was injected intravenously in the control and 7- and 9-wk groups and biodistribution in the heart, lung, spleen and blood were measured 4 hr postinjection with an automatic well-type gamma counter.

Exocytotic release and nonexocytotic metabolic release in adriamycin-induced heart failure were evaluated from alterations of [^{125}I]MIBG accumulations by treatment of the alpha-2 agonist, guanabenz (3 mg/kg i.p.), which inhibits exocytotic release, and by treatment of the uptake-1 blocker, desipramine (10 mg/kg i.p.), which inhibits nonexocytotic metabolic release. Guanabenz treatment was performed 1 hr before intravenous administration of [^{125}I]MIBG. Desipramine treatment was performed 15 min postinjection of [^{125}I]MIBG. For the autoradiography studies, [^{125}I]MIBG (250 μCi/kg) was injected intravenously to each animal in the control group and 9-wk groups under no treatment, guanabenz treatment and desipramine treatment, and the heart was removed 4 hr later. The results of pharmacologic perturbations were confirmed by autoradiographs which were analyzed using a computer-assisted image processing system.

TABLE 1
Mortality

	No. of rats	Mortality (%)
Controls	49	0
Adriamycin		
7 wk	49	4
8 wk	56	7
9 wk	77	26*

*$p < 0.005$ compared to controls.

Tritiated norepinephrine (25 μCi/kg) was injected intravenously in the control group and the 8-wk groups under no treatment and pretreatment with desipramine (10 mg/kg i.p. 1 hr before i.v. of ^{3}H-norepinephrine). Cardiac ^{3}H-norepinephrine uptake was measured 15 min postinjection in a liquid scintillation counter after tissue oxidation. The uptake-1 component was identified as the percent difference of cardiac ^{3}H-norepinephrine uptake produced by pretreatment with desipramine compared to control values under no pretreatment. Uptake-1 function was evaluated by comparison of the uptake-1 component between the control and 8-wk groups.

Plasma and myocardial concentration of endogenous norepinephrine were measured by high-performance liquid chromatography (HPLC). The norepinephrine precursor, dihydroxyphenylalanine (L-dopa), concentration in the plasma pool was measured as an indicator of norepinephrine synthesis by adrenergic neurons.

Statistical Analysis

All results were expressed as mean ± s.d. The experimental groups were compared by unpaired t-tests with corrections for multiple comparisons. A p value less than 0.05 was used to define statistical significance.

RESULTS

No mortality occurred in the control group, but 8% of the rats in the 7-wk group, 13% in the 8-wk group and 34% in the 9-wk group died during the treatment period (Table 1). Body and heart weight were decreased in the adriamycin groups. Pleural effusions and ascites began to appear in the 8-wk group and were observed frequently and massively in the 9-wk group (Table 2).

Radionuclide Angiography

Compared to the control group (81.2% ± 2.2%) LVEF showed dose-dependent decrease: 66.4% ± 4.9% in the 7-wk, 58.6% ± 5.5% in the 8-wk and 44.8% ± 10.1% in the 9-wk groups, respectively $p < 0.001$.

Plasma and Myocardial Norepinephrine Concentration

Plasma norepinephrine concentration significantly increased in the adriamycin groups (774 ± 636 pg/ml at 7 wk, 1060 ± 422 pg/ml at 8 wk and 1124 ± 515 pg/ml at 9 wk) compared to the control group (124 ± 30 pg/ml) and myocardial norepinephrine concentration significantly decreased in the adriamycin groups (490 ± 76 ng/g at 7 wk,

TABLE 2
Necropsy Results

	No. of rats	Body weight (g)	Heart weight (g)	Pleural effusion	Ascites
Control	31	403 ± 29	0.92 ± 0.07	0/31	0/31
Adriamycin					
7 wk	31	344 ± 18‡	0.94 ± 0.04	0/31	0/31
8 wk	36	334 ± 23‡	0.83 ± 0.07‡	2/36	7/36*
9 wk	31	327 ± 36‡	0.79 ± 0.07‡	17/31‡	19/31†

*$p < 0.01$; †$p < 0.005$; ‡$p < 0.001$ compared to controls.

493 ± 176 ng/g in 8 wk and 389 ± 71 ng/g at 9 wk) compared to the control group (734 ± 58 ng/g) (Fig. 2). Plasma dopa levels significantly increased at 7 (900 ± 255 pg/ml, $p < 0.02$) and 8 wk (1100 ± 316 pg/ml, $p < 0.01$) compared to controls (520 ± 130 pg/ml), but there was no difference between the control and 9-wk groups (750 ± 300 pg/ml, ns).

Iodine-125-MIBG Biodistribution

Cardiac MIBG accumulation decreased to 53% of control in the 7-wk group and markedly decreased to 14% of control in the 9-wk group (0.49% ± 0.06% kg dose/g in the control, 0.26% ± 0.04% kg dose/g in the 7-wk and 0.07% ± 0.01% kg dose/g in the 9-wk groups) (Fig. 3). Reduction of MIBG accumulation in the lung (0.24% ± 0.01% kg dose/g in the control, 0.23% ± 0.01% kg dose/g in the 7-wk and 0.15% ± 0.02% kg dose/g in the 9-wk groups) and spleen (0.20% ± 0.01% kg dose/g in the control, 0.21% ± 0.03% kg dose/g in the 7-wk and 0.15% ± 0.01% kg dose/g in the 9-wk groups) was less pronounced compared to reduction in the heart, although these are adrenergic-rich organs similar to the heart. MIBG levels in the blood increased dose-dependently (0.020% ± 0.004% kg dose/g in the control, 0.046% ± 0.014% kg dose/g in the 7-wk and 0.067% ± 0.007% kg dose/g in the 9-wk groups).

Cardiac Uptake of Tritiated Norepinephrine

There were no differences in cardiac uptake of ^{3}H-norepinephrine between the control and 8-wk groups (1.11% ± 0.10% kg dose/g compared to 0.98% ± 0.13% kg dose/g, ns) (Fig. 4). Cardiac uptake of ^{3}H-norepinephrine was decreased 91.0% in the control (0.10% ± 0.04% kg dose/g) and 90.8% in the 8-wk groups (0.09% ± 0.01% kg dose/g) by desipramine pretreatment, indicating no difference in the uptake-1 component.

Pharmacologic Perturbations

Treatment with guanabenz (i.p. 1 hr before MIBG i.v.) significantly increased myocardial MIBG values, from 0.49% ± 0.06% kg dose/g to 0.57% ± 0.06% kg dose/g ($p < 0.02$) in the control group and from 0.26% ± 0.04% kg dose/g to 0.48% ± 0.07% kg dose/g ($p < 0.001$) in the 7-wk group, and restored the 7-wk group value to almost that of the control animals not receiving guanabenz (Fig. 5). Although guanabenz treatment in the 9-wk group markedly increased myocardial MIBG values (from 0.07% ± 0.01% kg dose/g to 0.39% ± 0.08% kg dose/g ($p < 0.001$), yet significant reduction ($p < 0.05$) was observed compared to control values.

Treatment with desipramine (i.p. 15 min after MIBG i.v.) slightly decreased myocardial MIBG values in the control

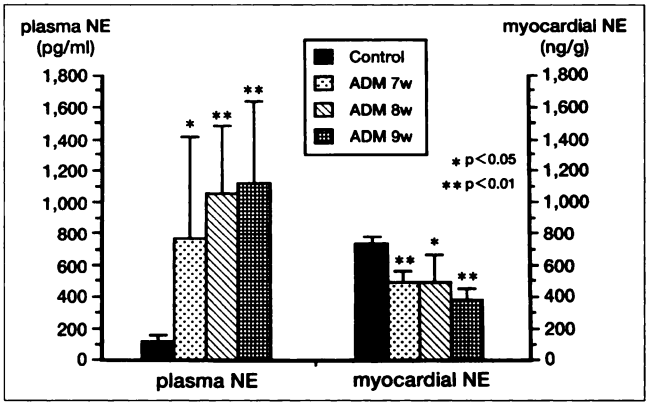

FIGURE 2. Plasma and myocardial norepinephrine (NE) concentrations. Plasma NE significantly increased in the adriamycin groups and myocardial NE significantly decreased compared to the controls.

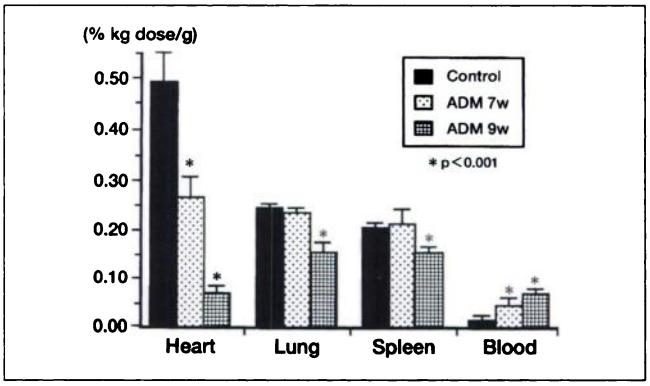

FIGURE 3. Iodine-125-MIBG accumulation (4-hr values). Cardiac MIBG accumulation decreased to 53% of controls in the 7-wk group and markedly decreased to 14% of controls in the 9-wk group. Reduction of MIBG accumulation in the lung and spleen was less pronounced compared to reduction in the heart. MIBG levels in the blood increased dose-dependently.

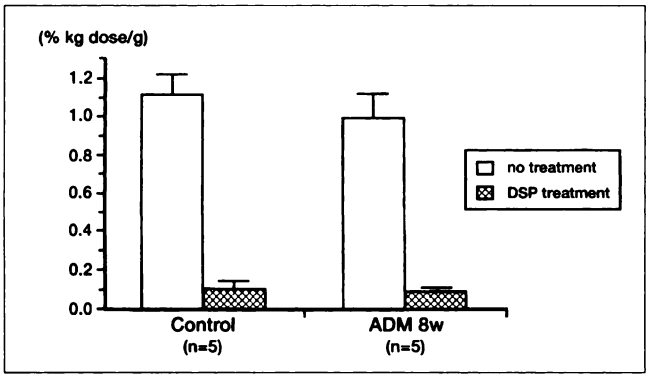

FIGURE 4. Cardiac uptake of ^{3}H-norepinephrine (NE) (15-min values). There was no difference in cardiac uptake of ^{3}H-NE between controls and the 8-wk group. Cardiac uptake of ^{3}H-NE was decreased 91% in the controls and 90.8% in the 8-wk group that had pretreatment with desipramine (DSP), indicating no difference in the uptake-1 component.

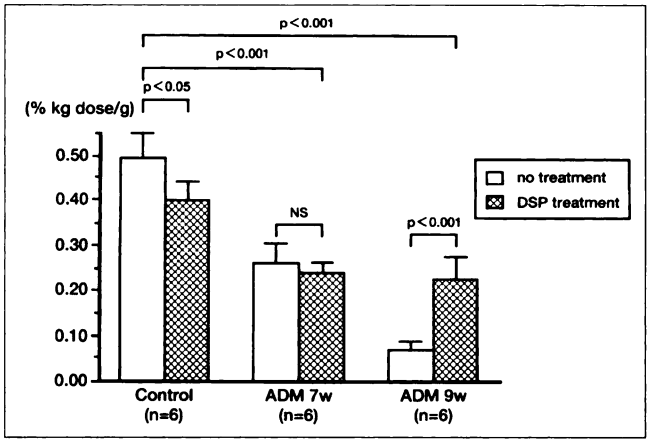

FIGURE 6. Change in myocardial [^{125}I]MIBG accumulation (4-hr values) after treatment with desipramine (DSP). DSP treatment slightly decreased myocardial MIBG values in the control and 7-wk groups, but significantly increased myocardial MIBG values in the 9-wk group.

group, from 0.49% ± 0.06% kg dose/g to 0.40% ± 0.04% kg dose/g ($p < 0.05$) and from 0.26% ± 0.04% kg dose/g to 0.24% ± 0.02% kg dose/g (p = ns) in the 7-wk group, but significantly increased myocardial MIBG values from 0.07% ± 0.01% kg dose/g to 0.22% ± 0.05% kg dose/g ($p < 0.001$) in the 9-wk group (Fig. 6).

Guanabenz treatment did not change MIBG values in the lung and spleen in the control and 7-wk groups and increased these values in the 9-wk group [from 0.15% ± 0.02% kg dose/g to 0.24% ± 0.03% kg dose/g ($p < 0.001$, in the lung) and from 0.15% ± 0.01% kg dose/g to 0.20% ± 0.02% kg dose/g ($p < 0.001$, in the spleen)], although less pronouncedly compared to in the heart. Desipramine treatment decreased MIBG values in the lung from 0.24% ± 0.01% kg dose/g to 0.19% ± 0.02% kg dose/g ($p < 0.001$) in the control group and from 0.23% ± 0.01% kg dose/g to 0.18% ± 0.03% kg dose/g ($p < 0.01$) in the 7-wk group.

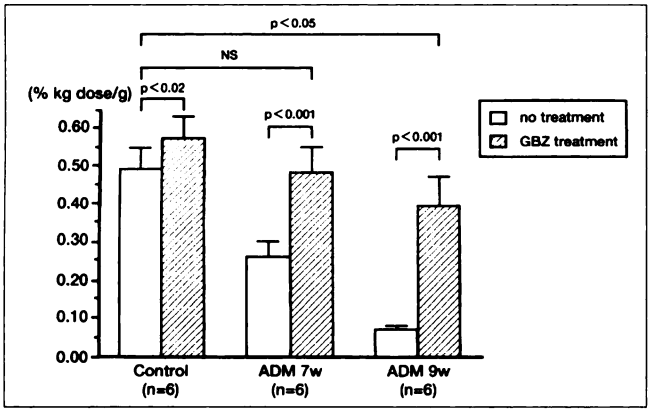

FIGURE 5. Change in myocardial [^{125}I]MIBG accumulation (4-hr values) after treatment with guanabenz (GBZ). GBZ treatment significantly increased myocardial MIBG values in the control and 7-wk groups and restored the 7-wk group value to almost that of the control value. Although GBZ treatment in the 9-wk group markedly increased myocardial values, significant reduction was observed compared to control values.

There was no significant change in the 9-wk group (from 0.15% ± 0.02% kg dose/g to 0.17% ± 0.03% kg dose/g, p = ns). Desipramine treatment decreased MIBG values in the spleen in the control, 7-wk and 9-wk groups: from 0.20% ± 0.01% kg dose/g to 0.13% ± 0.02% kg dose/g ($p < 0.001$), from 0.21% ± 0.03% kg dose/g to 0.09% ± 0.02% kg dose/g ($p < 0.001$) and from 0.15% ± 0.01% kg dose/g to 0.11% ± 0.02% kg dose/g ($p < 0.01$), for the three groups, respectively.

MIBG values in the blood did not significantly change in the control and in the 7-wk groups that had guanabenz and desipramine treatment; there was a decrease in the 9-wk group, from 0.067% ± 0.007% kg dose/g to 0.042 ± 0.007% kg dose/g ($p < 0.001$), due to guanabenz treatment and a return to the control value due to desipramine treatment (from 0.067% ± 0.007% kg dose/g to 0.023% ± 0.004% kg dose/g, $p < 0.001$).

Iodine-125-MIBG Autoradiography

Autoradiographs from the 9-wk group showed extensive marked decrease of [^{125}I]MIBG accumulation in the right and left ventricular walls (Fig. 7). Guanabenz treatment markedly increased [^{125}I]MIBG accumulation in the 9-wk group. Desipramine treatment decreased [^{125}I]MIBG accumulation in the control group but dramatically increased [^{125}I]MIBG accumulation in the 9-wk group.

DISCUSSION

In congestive heart failure, overall adrenergic nervous system activity is increased (*1–5*), but assessment of cardiac adrenergic activity in congestive heart failure has provided as many conflicting results as there are techniques available to measure it. We used radiolabeled MIBG to delineate the underlying neural pathophysiology in congestive heart failure due to adriamycin cardiomyopathy.

Cardiac MIBG accumulation, determined 4 hr after intravenous administration of MIBG, markedly decreased

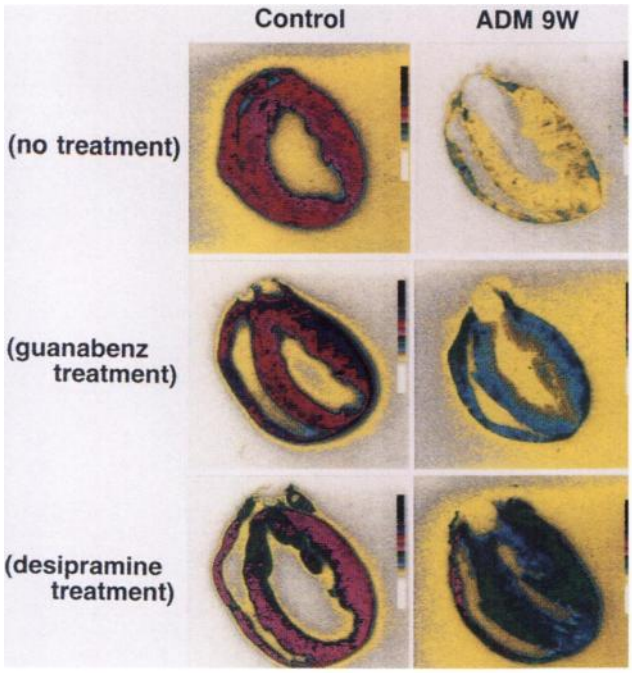

FIGURE 7. Iodine-125-MIBG autoradiography. Autoradiograph in the 9-wk group shows extensively marked decrease of [^{125}I]MIBG accumulation in the RV and LV walls. Guanabenz (GBZ) treatment markedly increased [^{125}I]MIBG accumulation in the 9-wk group. Desipramine (DSP) treatment decreased [^{125}I]MIBG accumulation in the control group but dramatically increased [^{125}I]MIBG accumulation in the 9-wk group.

with dose-dependent exacerbation of LVEF consistently. When the time course of MIBG accumulation in the vesicles at the adrenergic neuron terminals was examined to clarify the time when myocardial MIBG accumulation best reflects cardiac adrenergic activity, extravesicular accumulation rapidly decreased after intravenous administration, while intravesicular accumulation was relatively constant postinjection to reach a plateau 4 hr later (*20*). Our recent experiments using MIBG with a high specific radioactivity revealed accumulation of 80%–90% in cardiac neurons 3 hr postinjection (dose: 0.2–1.0 μg/kg) and storage of 70%–80% in adrenergic vesicles. Therefore, myocardial MIBG accumulation 4 hr postinjection reflects cardiac adrenergic neuron activity.

Decreased cardiac MIBG accumulation may be due to either increased neuronal release of MIBG (and hence, cardiac adrenergic hyperactivation) or to impaired cardiac neuronal uptake of MIBG (uptake-1). When the uptake-1 component was identified as the percent difference of cardiac ^{3}H-norepinephrine uptake produced by pretreatment with and without desipramine there was no difference in the uptake-1 component between the control and 8-wk groups. We believe there was no impairment in the capacity for neuronal uptake until the 8-wk group. Furthermore, treatment with guanabenz markedly increased cardiac MIBG values and restored them to approximately the control value in the 7-wk group. These findings suggest that decreased cardiac MIBG accumulation is produced by cardiac adrenergic hyperactivation (accelerated exocytotic release) until 8 wk in which heart failure was not advanced, although increased exocytosis might be demonstrated more convincingly by MIBG accumulation data on several time points.

Reduced MIBG accumulation in the lung and spleen was less pronounced compared to reduction in the heart and increased MIBG values by guanabenz treatment was only mild in both the lungs and spleen. It is possible that local cardiac adrenergic activation is predominant over systemic adrenergic activation in heart failure due to adriamycin cardiomyopathy. Bristow et al. (*21*) recently invesigated isolated right ventricular failure due to primary pulmonary hypertension and found that beta-adrenergic neuroeffector abnormalities in the failing human heart are produced by local rather than systemic mechanisms. Although guanabenz treatment at 9 wk markedly increased cardiac MIBG values, significant reduction was observed.

Nonexocytotic metabolic release via the uptake-1 carrier in reverse of normal transport direction has been demonstrated under conditions of energy depletion in myocardial ischemia, anoxia or cyanide intoxication (*15*). In our previous study (*22*), it was shown that both glucose and fatty acid utilization are decreased in adriamycin cardiomyopathy and these critical impairments in energy metabolism are associated with heart failure. In the present study, L-dopa concentration in the plasma pool, as an indicator of norepinephrine synthesis by adrenergic neurons, significantly increased in the 7- and 8-wk groups compared to controls, but there was no difference between controls and the 9-wk group. Reduced norepinephrine synthesis was suggested in the 9-wk compared to 7- and 8-wk groups.

Blockage of the uptake-1 carrier by tricyclic antidepressants such as desipramine inhibits both inward and outward transport and thus effectively suppresses nonexocytotic metabolic release. When desipramine treatment was performed 15 min after MIBG administration, cardiac MIBG values slightly decreased in the control and 7-wk groups. Since desipramine treatment was performed 15 min after MIBG administration, it is considered that uptake-1 mediated reuptake of MIBG released from the neurons was blocked in the control and 7-wk groups. When desipramine treatment was performed 15 min after MIBG injection in the 9-wk group, which had high mortality and massive pleural effusion and ascites, cardiac MIBG values dramatically increased. Desipramine treatment did not increase MIBG values in both the lung and spleen, even in the 9-wk group. These results may indicate that nonexocytotic metabolic release has been induced in cardiac adrenergic neurons due to energy depletion and different release mechanisms (exocytotic release and non-exocytotic local metabolic release) are active in parallel in the 9-wk group. Heart weight was reduced in the 8- and 9-wk groups, possibly because adrenergic neuron density would be increased in the heart of these groups. The chance of underestimating reduced ^{3}H-norepinephrine uptake and overestimating exocytotic release from the neuron should be considered.

The uptake-1 component, however, indicated no difference between the control and 8-wk groups. Reduction of heart weight in these groups was not so marked compared to controls, and marked reduction of MIBG values in these groups cannot be explained only by alterations of neuron density. Since we did not examine cardiac ^{3}H-norepinephrine uptake in the 9-wk group, it is uncertain whether neuronal uptake function is impaired at 9 wk. It may be assumed that exacerbation of energy depletion will result in failure of neuronal uptake and ultimately in disruption of cardiac adrenergic neurons' integrity (denervation). These findings on pathophysiology of cardiac adrenergic neurons in congestive heart failure in a rat model with adriamycin cardiomyopathy may be extrapolated to cases of clinical heart failure, such as coronary artery disease, dilated cardiomyopathy or valvular disease. Further studies are required to validate these possibilities.

CONCLUSION

Our results suggest that:

1. Congestive heart failure due to adriamycin cardiomyopathy progressively accelerates exocytotic release of norepinephrine predominantly from cardiac adrenergic neurons, but neuronal uptake function is not disturbed so long as heart failure is not advanced.
2. In the advanced stage, nonexocytotic metabolic release is induced specifically in cardiac adrenergic neurons due to energy depletion and norepinephrine release markedly increases.

Although it has been reported that chronic activation of adrenergic nervous system in congestive heart failure is accompanied by attenuation of feedback inhibition due to baroreceptor dysfunction (*22–24*), our present study indicates that local cardiac adrenergic activation is more pronounced than systemic adrenergic activation, and modulation of norepinephrine release in regional cardiac adrenergic neurons plays an important role for sustained adrenergic hyperactivation.

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