Discrepant Recovery Course of Sympathetic Neuronal Function and β-Adrenoceptors in Rat Hearts After Reperfusion Following Transient Ischemia

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Cardiac sympathetic neuronal function is closely coupled with β-adrenoceptors and adrenergic signaling. However, the recovery process of sympathetic neuronal function and β-adrenoceptors after reperfusion following transient ischemia is not fully understood. Accordingly, this study was performed to investigate serial changes in sympathetic neuronal function and β-adrenoceptors after transient myocardial ischemia. **Methods:** The left coronary artery of male Wister rats was ligated for 15 min followed by reperfusion. A dual-tracer method of ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG) and ^{125}I-iodocyanopindolol (^{125}I-ICYP) was used to assess cardiac sympathetic neuronal function and β-adrenoceptor density on days 1, 3, 7, 14, and 28 after reperfusion. Myocardial norepinephrine (NE) content in ischemic regions (IR) and in remote regions (RR) and hemodynamic indices were determined. Using a membrane preparation of the rat heart after reperfusion, the maximum specific binding (B_{max}) of β-adrenoceptors was compared with ^{125}I-ICYP accumulation. **Results:** The maximum value of the rate of change in left ventricular (LV) pressure (dP/dt_{max}) tended to decrease on day 1 after reperfusion but recovered thereafter. Myocardial NE content was significantly reduced in IR compared with RR on day 1 (272 ± 49 vs. 487 ± 93 ng/g, $P < 0.01$), and the decrease became more severe on day 14 (36 ± 19 vs. 489 ± 132 ng/g, $P < 0.01$) and day 28 (37 ± 14 vs. 455 ± 216 ng/g, $P < 0.01$). Decrease in the IR-to-RR uptake ratio of ^{131}I-MIBG was modest on day 1 (0.64 ± 0.12) and became more severe on days 7 and 14 (0.38 ± 0.12 and 0.35 ± 0.13, respectively). This reduction was partially restored on day 28 (0.50 ± 0.18). In contrast, the IR-to-RR uptake ratio of ^{125}I-ICYP was severely decreased until day 3 (0.60 ± 0.13 on day 1 and 0.54 ± 0.19 on day 3) and recovered thereafter. On day 3, B_{max} was significantly lower in IR than in RR (83 ± 17 vs. 100 ± 12 fmol/mg, $P < 0.05$), but the dissociation constant did not differ between the 2 regions. **Conclusion:** The recovery course of cardiac ^{131}I-MIBG uptake after reperfusion following transient ischemia is quite different from that of ^{125}I-ICYP. Simultaneous scintigraphic portrayal of β-adrenoceptors together with ^{131}I-MIBG would provide useful information regarding adrenergic system signaling in patients with coronary artery disease.

Key Words: myocardial ischemia; reperfusion; sympathetic nervous system; β-adrenergic receptor; radioisotopes

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During prolonged ischemia, nonexocytotic norepinephrine (NE) release from the sympathetic nerve terminal results in a marked increase in NE concentration in the synaptic cleft (*1–3*). Myocardial ischemia itself may cause impairment of cardiac sympathetic nerve function. In both clinical and animal studies, regional sympathetic dysinnervation was observed after myocardial ischemia associated with (*4–7*) and without (*8,9*) myocardial infarction (MI). In our previous study of rat MI model using a radiotracer of ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG) (*7*), an analog of NE, sympathetic neuronal dysfunction was detected in the region exceeding myocardial necrosis, and this expanded neuronal damage in the periinfarct region recovered 4 wk after the induction of MI. However, there are few reports of serial changes in sympathetic neuronal dysfunction after reperfusion following transient myocardial ischemia.

Sympathetic neuronal function is closely coupled with β-adrenoceptors and their signaling (*10,11*). The influences of ischemia on β-adrenoceptor density are still controversial. Some studies have shown that myocardial ischemia increased the number of β-adrenoceptors without affecting its affinity (*12,13*). This increase during ischemia might be induced by redistribution of β-adrenoceptors from the intracellular vesicles to the sarcolemmal fraction (*12*). After coronary reperfusion, the number of β-adrenoceptors decreased in the ischemic region (*14–16*) but increased, especially in the very early stage after reperfusion (*17*). Thus,

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serial changes in the number of β-adrenoceptors after reperfusion remain unresolved.

Recently, cardiac sympathetic neuronal function has been evaluated in patients with ischemic heart disease (*4,9,18*) and heart failure (*19–21*). Sympathetic neuronal function may directly affect β-adrenoceptors and their signaling (*11,22*); therefore, simultaneous assessment of sympathetic neuronal function and β-adrenoceptors may provide insight into the pathophysiology of various cardiac disorders. Using a dual-tracer method to assess sympathetic neuronal function with ^{131}I-MIBG and β-adrenoceptor density with ^{125}I-iodocyanopindolol (^{125}I-ICYP), this study was designed to elucidate serial changes in cardiac sympathetic neuronal function and β-adrenoceptor density after reperfusion following transient coronary occlusion.

MATERIALS AND METHODS

Experimental Animals

The experimental procedures followed the approved guidelines for animal experimentation at Toyama Medical and Pharmaceutical University.

Male Wistar rats weighting 300–350 g were used for induction of myocardial ischemia as described previously (*7*). Briefly, a left thoracotomy was performed to exteriorize the heart rapidly under ether anesthesia. The left coronary artery was ligated 2~3 mm from its origin with a suture of 5–0 Prolene (Ethicon, Inc.) for 15 min, and then the ligation was released because coronary artery occlusion lasting <15 min did not result in myocardial necrosis in our preliminary study. Standard rat chow and tap water were given ad libitum throughout the experiment.

The rats were divided into 5 groups. The first group (n = 19) was used for hemodynamic study. The second group (n = 23) was used for measurements of plasma and cardiac tissue catecholamines. The third group (n = 37) was used for cardiac autoradiography to evaluate ventricular distribution and accumulation of ^{131}I-MIBG and ^{125}I-ICYP. The fourth group (n = 10) was used for ^{201}Tl-chloride (^{201}Tl) accumulation, and the final group (n = 7) was used for determination of β-adrenoceptor density in membrane preparation. Age-matched, sham-operated rats (n = 41) were used for hemodynamic study and catecholamine measurement. A sham operation was performed using the same method as that used for the ischemia–reperfused rats, except for the coronary artery ligation. Data were collected on days 1, 14, and 28 after reperfusion for hemodynamic study and catecholamine measurement; on days 1, 3, 7, 14, and 28 for ^{131}I-MIBG and ^{125}I-ICYP autoradiographic study; and on days 1 and 7 for ^{201}Tl study. The study using the membrane preparation was performed on day 3 after reperfusion.

Hemodynamic Study

A 2-French micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery and advanced into the left ventricle (LV) to measure LV pressure. With the rat anesthetized lightly and breathing spontaneously, LV pressure and electrocardiograms were recorded on a multichannel thermal recorder (WT645G; Nihon Kohden). These signals were digitized online at 2-ms intervals and analyzed with a signal-processing computer system (7T-18; NEC San-Ei).

Measurement of Catecholamines

Blood was drawn from the carotid artery for an analysis of plasma catecholamines under ether anesthesia. An overdose of sodium pentobarbital (70 mg/kg) was then injected intraperitoneally. The chest was opened and the heart was removed quickly. The right ventricle (RV) and LV were dissected and rinsed in ice-cold saline. The nonischemic, remote region (RR) of the interventricular septum and the ischemic region (IR) of the LV free wall were cut and prepared for measurement of tissue catecholamines. The IR was determined as the region distal to the occlusion site. Plasma and tissue samples were stored at −80°C for later analyses. Catecholamine concentrations were determined by automated high-performance liquid chromatography (HPLC) as described previously (*11*).

Radiopharmaceuticals

^{131}I-MIBG had a radiochemical purity of >98% and a specific activity of 7.4 GBq/mg. The structure of ^{125}I-ICYP is shown in Figure 1. L-^{125}I-ICYP was synthesized by a modified chloramine-T radioiodination of L-cyanopindolol followed by a reversed-phase HPLC purification (*23*). ^{125}I-ICYP had a radiochemical purity of >97% and a specific activity of >75 TBq/mmol. ^{131}I-MIBG and ^{125}I-ICYP were prepared at Daiichi Radioisotope Laboratory. (−)-4-(3-*t*-Butylamino-2-hydroxypropoxy)-[5,7-^{3}H]benzimidazol-2-one (^{3}H-CGP12177) was purchased from New England Nuclear, Inc.

Dual-Tracer Autoradiography

Dual-tracer autoradiography was performed as described previously (*11*). Briefly, 1.85 MBq ^{131}I-MIBG were injected via the external jugular vein under anesthesia with sodium pentobarbital (30 mg/kg intraperitoneally). Two hours later, 0.37 MBq ^{125}I-ICYP were given intravenously. The heart was removed 1 h after the injection of ^{125}I-ICYP and washed in cold saline. The specimens were frozen in isopentane, cooled in dry ice, and embedded in methyl cellulose followed by preparation of serial 20-μm-thick transverse sections. Four days after the radiotracer injection, the first autoradiographic exposure on an imaging plate (BAS-UR; Fuji Film) was performed for 6 h to reveal ^{131}I-MIBG distribution. The second exposure was initiated 75 d later after the decay of ^{131}I-MIBG activity and required 28 d for adequate image quality. A myocardial section at a level of papillary muscle was used for the quantification of myocardial distribution of ^{131}I-MIBG and ^{125}I-ICYP. In single-tracer autoradiography—with each tracer under the same condition as that of the dual-tracer method—it was confirmed that ^{125}I-ICYP density was <3% of ^{131}I-MIBG density under the condition of exposure for ^{131}I-MIBG imaging and that ^{131}I-MIBG density was <2% of ^{125}I-ICYP density under the condition of exposure for ^{125}I-ICYP imaging. Therefore, the cross-talk between ^{125}I and ^{131}I could be negligible.

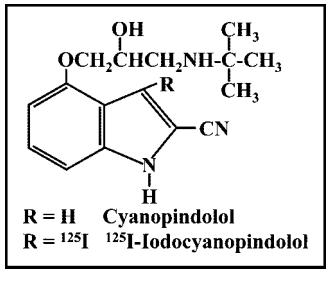

FIGURE 1. Schematic illustrations of cyanopindolol.

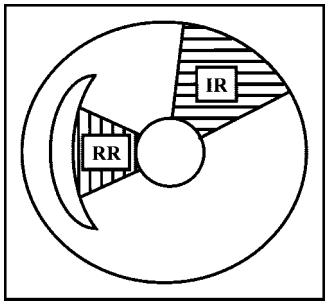

FIGURE 2. Schematic diagram for autoradiographic quantification of myocardial accumulation of ^{131}I-MIBG and ^{125}I-ICYP. Two regions of interest were determined.

To evaluate the myocardial accumulation and distribution of ^{131}I-MIBG and ^{125}I-ICYP, the autoradiographic images were analyzed using a computer-assisted imaging–processing system (BAS3000; Fuji Film) as described previously (*11*). As shown in Figure 2, a region of interest (ROI) was put on the LV anterior wall (IR) and the interventricular septum (RR) at the level of papillary muscles. The IR was determined referring to our previous study of rat MI (*7*), in which the same coronary artery occlusion method, except for reperfusion, was used and its infarct size was 35% of the LV. Therefore, the IR in this study was estimated on the autoradiographic image as one sixth of the whole LV area around the center of ischemia to avoid the nonischemic or border zone. The myocardial tracer uptake in the IR and RR was normalized as a percentage of the administered dose per gram of heart (% kg dose/g), using ^{131}I- and ^{125}I-labeled graded standards—that is, 20-μm-thick sections of methyl cellulose, including 4 different concentrations of each tracer, were prepared and exposed on the imaging plate simultaneously with the heart preparations.

^{201}Tl Autoradiography

Quantitative ^{201}Tl autoradiography was performed to estimate myocardial blood perfusion after reperfusion following transient ischemia on days 1 and 7. Briefly, 9.25 MBq ^{201}Tl were injected via the external jugular vein. The heart was removed 5 min after ^{201}Tl injection. The autoradiographic exposure was performed for 3 h to reveal ^{201}Tl distribution. Quantification of myocardial ^{201}Tl uptake was performed using a method similar to that of the ^{131}I-MIBG and ^{125}I-ICYP study. ^{201}Tl graded standards were prepared by the same method as those of ^{131}I-MIBG and ^{125}I-ICYP.

β-Receptor Binding Assay

The method for determination of the maximum specific binding (B_{max}) of β-adrenoceptors was reported previously (*11*). Briefly, under pentobarbital anesthesia (70 mg/kg intraperitoneally), hearts were quickly removed and rinsed in saline at 4°C. Tissue membrane preparations obtained from IR and RR were incubated with ^{3}H-CGP12177 at 37°C for 60 min. The radioactivity trapped on the glass filters was counted in a scintillation counter with Aquazol II (Amersham). The nonspecific binding was defined as radioligand binding in the presence of an excess concentration (100 μmol/L) of DL-isoproterenol. Data from the saturation binding studies were analyzed by Scatchard analysis, giving the B_{max} and the dissociation constant (K_d).

Statistics

Data are expressed as mean ± SD. Group comparisons were made using ANOVA followed by the Bonferroni *t* test to identify differences between the various groups. $P < 0.05$ was considered statistically significant.

RESULTS

Hemodynamic Indices and Levels of NE

Hemodynamic indices are shown in Table 1. Heart rate and LV systolic pressure did not differ between rats with coronary ligation and rats with sham operation throughout the study periods. The maximum rate of change in LV pressure (dP/dt_{max}) in rats with coronary occlusion tended to decrease on day 1 after reperfusion but recovered thereafter. In both groups, plasma NE concentrations were highest on day 1, probably due to the surgical procedure. Myocardial NE contents in the IR significantly decreased on day 1 after reperfusion and decreased much more thereafter, whereas there were few changes in the NE contents throughout the study periods in the RR (Fig. 3).

^{201}Tl Accumulation

The ^{201}Tl accumulation ratio of IR to RR was significantly decreased on day 1 (0.68 ± 0.11; $n = 5$) but was restored on day 7 (0.90 ± 0.08; $n = 5$).

^{131}I-MIBG Accumulation

Representative examples of autoradiography with ^{131}I-MIBG were shown in Figure 4. A modest reduction of ^{131}I-MIBG uptake was observed in the IR on day 1 after reperfusion, especially in the subendocardial region. On day 14, however, the reduction of ^{131}I-MIBG uptake became more marked in the IR and extended toward the RR. This

TABLE 1
Hemodynamic Data

Parameter	Day 1		Day 14		Day 28	
	I/R ($n = 6$)	SH ($n = 8$)	I/R ($n = 7$)	SH ($n = 6$)	I/R ($n = 6$)	SH ($n = 7$)
HR (bpm)	442 ± 31	416 ± 44	398 ± 36	393 ± 37	399 ± 51	371 ± 29
LVSP (mm Hg)	111 ± 8	117 ± 6	111 ± 8	117 ± 9	120 ± 6	112 ± 12
dP/dt_{max} ($\times 10^3$ mm Hg/s)	9.4 ± 1.1	11.5 ± 1.3	10.2 ± 1.6	11.0 ± 2.4	10.7 ± 1.6	10.4 ± 2.4
$-dP/dt_{min}$ ($\times 10^3$ mm Hg/s)	6.4 ± 0.9	7.6 ± 1.0	6.0 ± 1.0	7.2 ± 0.9	6.4 ± 1.0	6.5 ± 1.4

I/R = coronary occlusion-reperfusion rats; n = number of rats; SH = sham-operated rats; HR = heart rate in beats per minute; LVSP = LV systolic pressure; dP/dt_{max} and $-dP/dt_{min}$ = maximum and minimum values of rate of change in LV pressure.
Data are presented as mean ± SD.

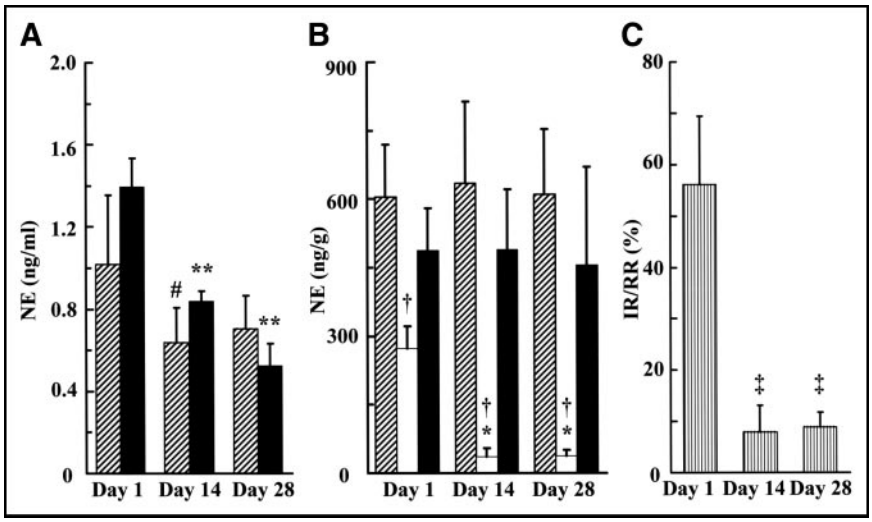

FIGURE 3. Levels of plasma (A) and myocardial (B) NE and myocardial NE ratio of IR to RR (C) in rats with transient coronary occlusion. (A) Hatched bars indicate sham-operated rats (n = 6, 5, and 6 on days 1, 14, and 28, respectively). Black bars indicate rats with coronary occlusion (n = 4, 6, and 7 on days 1, 14, and 28, respectively). (B) Hatched bars indicate sham-operated rats (n = 8, 6, and 6 on days 1, 14, and 28, respectively). White bars and black bars indicate IR and RR, respectively, in rats with coronary occlusion (n = 7, 9, and 7 on days 1, 14, and 28, respectively). Data are mean ± SD. **$P < 0.05$ vs. coronary occlusion rats on day 1; #$P < 0.05$ vs. sham-operated rats on day 1; †$P < 0.01$ vs. RR on each experimental day; *$P < 0.01$ vs. IR on day 1; ‡$P < 0.01$ vs. on day 1.

reduced ^{131}I-MIBG accumulation tended to recover on day 28. ^{131}I-MIBG accumulation in the IR decreased progressively until day 14 but partially recovered on day 28. The accumulation ratio of IR to RR was lowest between days 7 and 14 after reperfusion (Fig. 5).

^{125}I-ICYP Accumulation

Representative examples of autoradiography with ^{125}I-ICYP were shown in Figure 4. ^{125}I-ICYP accumulation on day 1 after reperfusion was decreased in the IR. On day 14, ^{125}I-ICYP accumulation became homogeneous in the whole LV. ^{125}I-ICYP accumulation in the IR was significantly decreased until day 3 and recovered thereafter (Fig. 6).

β-Receptor Binding in Membrane Preparation

The maximum specific binding of β-adrenoceptors (B_{max}) in the IR was significantly decreased compared with that in the RR on day 3 after reperfusion, whereas the K_d did not differ between the 2 regions (Table 2). This reduction of B_{max} in the IR was comparable with the results from the ^{125}I-ICYP accumulation study using dual-tracer autoradiography.

DISCUSSION

The major findings of this study are as follows. First, reduction in cardiac ^{131}I-MIBG accumulation induced by transient ischemia followed by reperfusion was relatively mild at 24 h after reperfusion and became gradually more marked until 2 wk later. One month later, however, it tended to recover. In contrast, ^{125}I-ICYP accumulation decreased only in the early stage after reperfusion and recovered 7 d later. Thus, serial changes in β-adrenoceptor density after reperfusion were quite different from those of sympathetic neuronal dysfunction. Second, this time course of neuronal dysfunction was comparable with changes in myocardial NE contents. Finally, assessment of β-adrenoceptor density

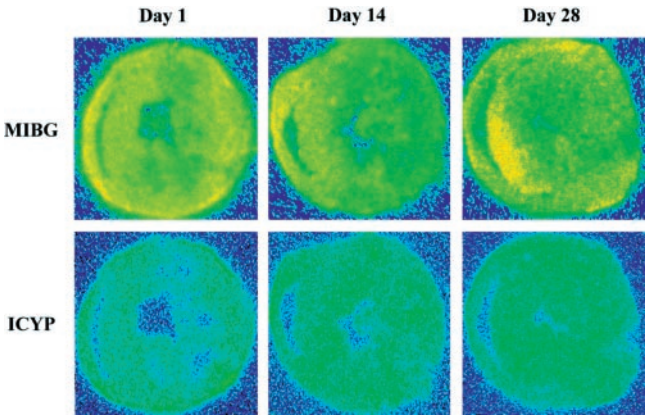

FIGURE 4. Representative examples of dual-tracer autoradiography with ^{131}I-MIBG (top) and ^{125}I-ICYP (bottom) on days 1, 14, and 28 after reperfusion following 15-min ischemia in rat hearts. Images were obtained from transverse section of hearts at level of papillary muscle. ^{125}I-ICYP images were derived from same slices that produced ^{131}I-MIBG images on each day.

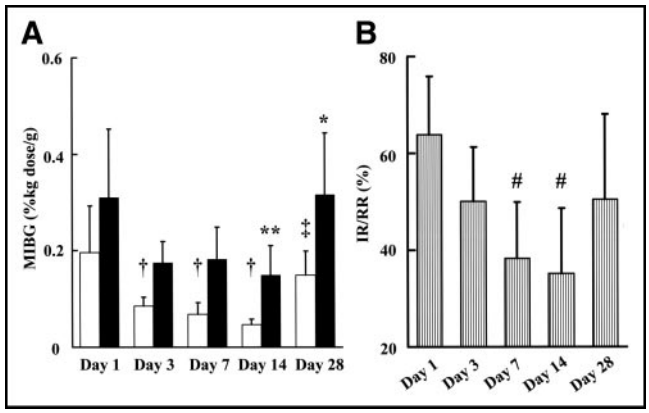

FIGURE 5. (A) Myocardial ^{131}I-MIBG accumulation of IR (white bars) and RR (black bars) (n = 7, 8, 7, 7, and 8 on days 1, 3, 7, 14, and 28, respectively). (B) ^{131}I-MIBG accumulation ratio of IR to RR. Data are mean ± SD. †$P < 0.01$ vs. IR on day 1; ‡$P < 0.01$ vs. IR on day 14; **$P < 0.05$ vs. RR on day 1; *$P < 0.05$ vs. RR on day 14; #$P < 0.05$ vs. on day 1.

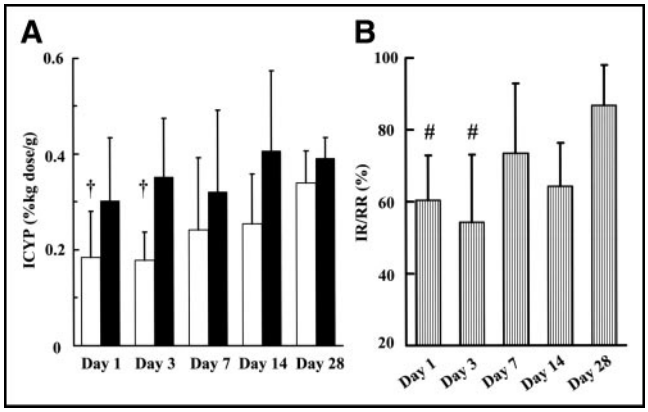

FIGURE 6. (A) Myocardial ^{125}I-ICYP accumulation of IR (white bars) and RR (black bars). Number of rats is same as in Figure 5. (B) ^{125}I-ICYP accumulation ratio of IR to RR. Data are mean ± SD. †$P < 0.05$ vs. IR on day 28; #$P < 0.05$ vs. on day 28.

obtained after intravenous injection of ^{125}I-ICYP was comparable with the data from a conventional radioligand binding assay in a membrane preparation; therefore, this method would be useful to determine ventricular distribution of β-adrenoceptors in ischemic hearts.

^{131}I-MIBG Accumulation

MIBG is an analog of NE and shares neuronal transport and storage mechanisms with NE (*24–26*). Extravesicular accumulation of MIBG at the adrenergic nerve terminal decreases rapidly after the injection and intravesicular accumulation reaches a plateau until 3 h after the injection (*24*). Serial changes in cardiac ^{131}I-MIBG accumulation in the IR after reperfusion were closely correlated with those of myocardial tissue NE content, except on day 28 when the decrease in ^{131}I-MIBG accumulation in the IR tended to recover despite lack of restoration of the myocardial NE contents. The ^{201}Tl autoradiography study suggested that the marked decrease in ^{131}I-MIBG accumulation 1–2 wk after reperfusion did not result from impaired coronary circulation, although ^{131}I-MIBG accumulation in the earlier stage might be affected, at least in part, by the coronary flow.

MIBG accumulation is considered to reflect the level of cardiac NE (*7,26,27*), a finding consistent with the present results. One month after reperfusion, however, partial recovery of ^{131}I-MIBG accumulation in the IR was not accompanied by restoration of the myocardial NE contents. Since the level of myocardial NE might be affected by various factors, including adrenergic neuron density, neuronal activity, uptake-1 function, and capacity of NE synthesis, a discrepancy between cardiac ^{131}I-MIBG accumulation and NE content could be detected under some disease conditions (*11,28*).

In patients with angina pectoris but without MI, cardiac MIBG uptake is often reduced in ischemic territory and, therefore, MIBG scintigraphy is considered to be useful to detect previous myocardial ischemic insult, especially in patients with vasospastic angina (*9,29*). However, there was little information on the time course of cardiac ^{131}I-MIBG accumulation after reperfusion following transient ischemia. In our previous study (*7*), ^{131}I-MIBG and ^{125}I-ICYP distributions in the LV were homogeneous in sham-operated rats. Surprisingly, reduction of ^{131}I-MIBG accumulation in the present study was more severe on days 7 and 14 than on day 1, and a long period >1 mo would be required for its full recovery. The progressive deterioration of ^{131}I-MIBG uptake after MI was observed in previous studies (*6,30*).

NE is released from sympathetic nerve terminals in response to myocardial ischemia (*1–3*). This NE release is mediated by 2 different mechanisms: an exocytotic release and a carrier-mediated nonexocytotic release. In the early period of ischemia (<10 min of ischemia), efferent sympathetic nerves are activated and NE is released by exocytosis (*2*). During more prolonged ischemia, as seen in this study, a large amount of NE might be released by a nonexocytotic mechanism and, hence, increase interstitial NE concentration (*1–3*). The released NE from sympathetic nerve terminals can be autoxidated, which in turn leads to the formation of cytotoxic hydroxyl free radicals (*31*). In a study by Liang et al. (*32*), impaired function of neuronal NE uptake induced by a continuous infusion of NE was prevented by antioxidant vitamins or superoxide dismutase. Thus, the sympathetic nerve terminals might be damaged by NE-derived free radicals (*32,33*). This NE-derived free radical formation after reperfusion may contribute to the progressive deterioration of ^{131}I-MIBG accumulation in the IR in the present study.

Cardiac ^{131}I-MIBG accumulation in the RR tended to decrease from day 3 to day 14 after reperfusion, although the decrease was only significant on day 14. Attenuation of ^{131}I-MIBG accumulation in the RR was also observed in previous studies of MI (*6,7*). In ischemia-reperfusion rat heart studied by Iwasaki et al. (*30*), ^{131}I-MIBG accumulation in the RR decreased more severely on day 2 after reperfusion than at 4 h. Although the exact mechanism of decreased ^{131}I-MIBG accumulation in the RR remains to be elucidated, an acute hemodynamic deterioration and subsequent activation of cardiac sympathetic nerves may result in an impaired cardiac neuronal uptake function or increased ^{131}I-MIBG clearance from the heart.

TABLE 2
β-Receptor Density and K_d in Membrane Preparations

Parameter	Day 3 (n = 7) IR	Day 3 (n = 7) RR
B_{max} (fmol/mg)	83 ± 17*	100 ± 12
K_d (nmol/L)	0.18 ± 0.05	0.17 ± 0.03

*$P < 0.05$ vs. RR.
n = number of rats.
Data are presented as mean ± SD.

^{125}I-ICYP Accumulation

ICYP has chirality and D-ICYP has no affinity for β-adrenoceptors. Specific binding of ^{131}I-pindolol in the rabbit ventricle was saturable, reversible, and stereospecific with a high affinity for the β-adrenoceptors but there was considerable nonspecific binding (*34*). An intravenous injection of ^{125}I-ICYP, however, bound predominantly to β-adrenoceptors, and nonspecific binding was 10%–20% of the total binding (*35*). In our previous study of rats (*11*), cardiac accumulation of L-^{125}I-ICYP injected intravenously increased linearly at doses from 0.185 to 5.55 MBq, and the increase was suppressed at a dose of 14.8 MBq, although the lung accumulation of ^{125}I-ICYP was greater than the cardiac accumulation. In the present study of ischemia-reperfusion hearts, reduction of ^{125}I-ICYP accumulation in the IR was comparable with decreases in B_{max} without significant changes in K_d, consistent with our previous results of hypertensive heart failure of rats (*11*). Therefore, the present method of intravenous injection of ^{125}I-ICYP could reasonably reflect changes in β-adrenoceptor density and might be useful for determination of β-adrenoceptor density and distribution in a living heart, although the lung accumulation of ^{125}I-ICYP should be resolved for its clinical application.

The effects of ischemia on β-adrenoceptor density remain controversial (*12*,*13*,*36*,*37*). These conflicting results appear to be due to different periods of ischemia and different degrees of reperfusion under different experimental conditions. In reperfusion models, however, many studies have reported a decrease in the B_{max} without changes in the affinity (*14–16*), a finding consistent with the present results, although Persad et al. (*16*) reported decreases in both the B_{max} and the affinity in a Langendorff preparation of rat heart with 30-min ischemia and 60-min reperfusion. Coronary reperfusion produced massive hydroxyl free radicals, which may play a role in decreasing the β-adrenoceptor density (*38*). Another explanation for a decrease in β-adrenoceptor density could be an interaction between sympathetic nerve function and β-adrenoceptors. During reperfusion after ischemia, a large amount of NE released from the sympathetic nerve terminals in association with concomitant dysfunction of NE reuptake would markedly increase levels of NE concentration in the synaptic cleft. Actually, synaptic NE levels were inversely related to β-adrenoceptor densities in dogs with pacing-induced heart failure (*22*). In our previous study of hypertensive heart failure of rats (*11*), increased sympathetic activity and impaired function of NE reuptake were associated with downregulation of β-adrenoceptors. Thus, increases in the levels of synaptic NE during ischemia and reperfusion might contribute to a decrease in β-adrenoceptor density.

Methodologic Limitations

Some methodologic limitations deserve comments in interpreting the present results. First, the reduction of ^{131}I-MIBG accumulation after coronary artery ligation could be a result of ligation of the nerve bundles accompanying the vessels rather than a result of ischemic damage. Holmgren et al. (*39*) reported that 5-h ligation around the nerve bundles and vein only, avoiding the coronary artery, produced no reduction of catecholamine fluorescence. Adrenergic nerve fibers run along the coronary arteries and are distributed in the perfusion area of the associated coronary artery (*39*). Myocardial ischemia induced either by intracoronary balloon inflation (*8*) or by intracoronary injection of microspheres (*5*) resulted in reduction of ^{131}I-MIBG uptake beyond the infarct region, a finding compatible with the present study. In a previous study by Iwasaki et al. (*30*) and us (*7*), the reduced area of ^{131}I-MIBG accumulation was more extensive than the perfusion area of the occluded artery. In our study of a 5-min ligation of the left coronary artery before prolonged ligation (30 min) in rats, reduction of ^{131}I-MIBG accumulation in the IR on day 3 after reperfusion was attenuated compared with that of the 30-min ligation only (0.062 ± 0.030 vs. 0.031 ± 0.011, % kg dose/g; $P < 0.05$). Using a microdialysis method (unpublished data), we found that this attenuation of ^{131}I-MIBG uptake induced by a brief episode of coronary ligation before prolonged ligation was associated with a smaller amount of NE release from the sympathetic nerve terminals during the prolonged ischemia. Taken together with the above observations, reduction of ^{131}I-MIBG accumulation due to the 15-min coronary occlusion in the present study resulted primarily from myocardial ischemia, although a direct sympathetic nerve injury due to coronary ligation could not be excluded.

Second, some amount of ^{131}I-MIBG injected may be taken up by nonneuronal tissue in the heart. Reduced ^{131}I-MIBG accumulation in the infarct region after coronary occlusion followed by reperfusion might result from a deficit in nonneuronal accumulation (*40*). However, nonneuronal accumulation of ^{131}I-MIBG could be washed out by 3 h after the injection (*24*).

CONCLUSION

Reduction of cardiac ^{131}I-MIBG accumulation was most severe between 1 and 2 wk after ischemia-reperfusion and was sustained for >4 wk, whereas the reduction of ^{125}I-ICYP was limited to only several days. Thus, ^{131}I-MIBG accumulation after ischemia-reperfusion was quite different from that of ^{125}I-ICYP. The present method of cardiac imaging with ^{125}I-ICYP and ^{131}I-MIBG could be useful for an in vivo evaluation of changes in cardiac adrenergic signaling after ischemic insult. This discrepant influence of ischemia-reperfusion on sympathetic neuronal function and β-adrenoceptors may contribute to the pathophysiology in ischemic hearts, such as desensitization of β-adrenoceptor signaling in the early stage after ischemia-reperfusion and denervation supersensitivity in the later stage, which may relate to arrhythmogenesis.

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