Effects of Cardiac Sympathetic Nervous System on the Stunned Myocardium
Experimental Study with \(^{123}\text{I}-\text{metaiodobenzylguanidine}\)

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\(^{123}\text{I}-\text{Metaiodobenzylguanidine (^{123}\text{I-MIBG}) uptake in the stunned myocardium was investigated in open chest dogs. ^{123}\text{I-MIBG is a tracer taken up in presynaptic adrenergic vesicles and reflects the function of the myocardial sympathetic nervous system. This study revealed that in the stunned myocardium without infarct, ^{123}\text{I-MIBG uptake was normal up to 40 minutes of ischemia and that exogenous noradrenaline improved deteriolated regional wall motion with increased uptake of ^{123}\text{I-MIBG. However, uptake of ^{123}\text{I-MIBG per flow decreased with infarct in ischemic areas, and it showed a linear relation with regional wall motion. Thus, in the absence of infarction ^{123}\text{I-MIBG is a tracer to differentiate stunning from more severe ischemia with persistent wall motion abnormality. Normal uptake and storage of ^{123}\text{I-MIBG in the stunned condition suggests that catecholamine release or second effector mechanism may relate to the mechanism.}}}

It is important to differentiate the irreversible myocardium from the reversible ischemic or stunned myocardium! The mechanism of stunning is still unclear, but the cardiac sympathetic nervous system has been suggested to be one of the important factors of stunning\(^5\)–\(^5\) Beta-blocker treatment was reported to be effective for the stunned myocardium\(^6\) but catecholamine infusion\(^7\) or beta-adrenergic stimulation\(^8\) was also effective for stunning. \(^{123}\text{I-Metaiodobenzylguanidine (MIBG) was taken up in the presynaptic storage vesicles of the adrenergic nerve and a clinical study was performed to evaluate the sympathetic nervous system.}\(^9\)–\(^\)\(^{12}\) Details of \(^{123}\text{I-MIBG uptake in cases with the stunned myocardium, however, have not been reported and, this paper is the first to evaluate \(^{123}\text{I-MIBG in the stunned myocardium.}}}

MATERIALS AND METHOD

Adult mongrel dogs of either sex, weighing 13 to 43 kg were subjected to an overnight fast and anesthetized with 10.0 mg/kg of ketaral i.m. and 25.0 mg/kg of sodium pentobarbital i.v. Animals were intubated and ventilated with room air, and the blood gas was kept within the physiological range. After an extravascular route was obtained, left thoracotomy was performed at the fifth intercostal space and the heart was suspended in a pericardial cradle. A segment of the left anterior descending coronary artery between the first and the second diagonal branch was gently dissected free and a doppler flow velocity probe and a snare were placed around the artery from

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Japanese Circulation Journal Vol.55, September 1991 893
proximal to distal order. A catheter was inserted into the left atrial appendage to inject isotope tracers.

**Experimental protocol**

After stabilization of the doppler flow, control recording of echocardiography was done with a 3.75 MHz transducer from the epicardium. Then the left anterior descending coronary artery (LAD) was ligated for a given period of time with a snare. Reperfusion of the ligated coronary artery was done with caution so as not to get reactive hyperemia compared to the control level. This usually requires more than 5 min to reach a steady level. $^{123}$I-MIBG in a dose of 22–26 MBq was injected through the intracardiac catheter. Echocardiographic recordings were taken just before reperfusion, 1 h, 2 h and 3 h after reperfusion. Subsequently, $^{123}$I-MIBG imaging was performed with a Searle Gamma camera and imaging was analyzed with a DEC computer (PDP-11/60). After that, $^{201}$Tl (37–74 MBq) was injected through the atrial catheter to obtain myocardial imaging.

Evans blue dye was injected through atrial catheter (10%, 100 cc) to outline an ischemic area just after ligation of LAD with the same snare as used previously. Saturated KCl was used to sacrifice the dog. The heart was excised and cut into 4 or 5 slices perpendicular to the long axis, in the same way as with the short axis used for echocardiographic recording.

Slices of the heart were placed serially before a gamma camera from apex to base for ex-vivo imaging with $^{123}$I-MIBG and $^{201}$Tl. Four hundred thousand counts were collected for in-vivo imaging and 200,000 counts for ex-vivo imaging. Non-stained areas with Evans blue dye were traced on a translucent paper to measure ischemic areas of LAD that were at risk. Slices of the heart were dipped in triphenyl-tetrazolium-chloride (TTC) with a solution of Sorensen's buffer for about 15 to 20 min. Non-stained areas with TTC were considered to be infarcted areas and were also traced on paper. Using staining as a reference, ten small pieces (0.5–1.0 gram per piece) of the myocardium were cut from the endocardium and the epicardium to examine the tissue count of $^{201}$Tl and $^{123}$I-MIBG.

**Echocardiographic evaluation of wall motion**

Regional wall motion was evaluated by regional shortening of the epicardium. The position of the transducer was kept constant throughout the study and the short axial image at the level of the papillary muscle was selected for calculation. Motion was measured along 100 chords constructed perpendicular to a centerline drawn midway between end-diastolic and end-systolic contours with a computer. The degree of shortening was calculated as a % of the control value and was expressed in a circumferential profile. Every chordal shortening in areas with shortening less than 50% of control was averaged as the value of the ischemic areas.

**Evaluation of ischemic and infarct area**

The area of ischemia or of infarct was measured by a planimeter and was expressed as a % of the corresponding whole surface area in each slice. Percentage area of both sides of the slice was averaged and multiplied by the weight of the slice. The total % of ischemic or infarct weight of left ventricle (LV) was added up.

**Evaluation of myocardial imaging and tissue count of both $^{123}$I-MIBG and $^{201}$Tl**

$^{201}$Tl uptake in ex-vivo imaging and tissue counts at ischemic areas was decreased with ligation for more than 40 min. Some of the in-vivo imaging with $^{123}$I-MIBG failed to show imaging defects, but ex-vivo imaging clearly delineated ischemic areas. Scintillation counts of peak energy were 159 keV for $^{123}$I-MIBG and 80 keV for $^{201}$Tl, with a window of 20%. Tissue counting was also done under the same conditions as the imaging for a minute in each sample with a well counter. Tissue counts in ischemic areas of the endocardium and the epicardium were averaged and compared to mean uptake of normal regions, and then expressed as relative uptake to normal areas (% uptake). Relative uptake of $^{123}$I-MIBG per unit flow obtained with $^{201}$Tl was calculated by an equation of $^{123}$I-MIBG (counts/gram of myocardium)/$^{201}$Tl (counts/gram of myocardium).

**Evaluation of regional shortening and $^{123}$I-MIBG uptake per flow after noradrenaline loading in stunning without infarct**

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TABLE 1

DOGS (open chest n=22)

<table>
<thead>
<tr>
<th></th>
<th>&lt;1 hour ischemia</th>
<th>≥1 hour ischemia</th>
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<tr>
<td>20 min OCC</td>
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<td>N=5</td>
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<tr>
<td>TTC (%)</td>
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<td>0.9 ± 0.5</td>
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<tr>
<td>EB (%)</td>
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<tr>
<td>40 min OCC</td>
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<tr>
<td>TTC (%)</td>
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<tr>
<td>EB (%)</td>
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<tr>
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<td>2 hours OCC</td>
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*OCC = occlusion, NA = Noradrenalin

Regional shortening with time after reperfusion and a ratio of $^{123}$I-MIBG/$^{201}$TI count was evaluated in stunning with noradrenaline infusion (2.5 μg/min) started after 1 hour of reperfusion following 40 min occlusion.

RESULTS

Twenty-two open chest dogs were analyzed in this study protocol. They were divided into 5 study groups; 5 dogs of 20 min occlusion with reperfusion (G-20), 5 dogs of 40 min occlusion with reperfusion (G-40) and three dogs of 40 min occlusion with noradrenaline infusion after reperfusion (G-40N), 4 dogs of 1 hour of occlusion with reperfusion (G-1H) and 5 dogs with 2 to 3 h of occlusion and reperfusion (G-2H). Infarct size delineated with TTC and ischemic area delineated with EB are shown in Table I. Sizes of ischemic areas were not significantly different among these groups, but infarct area was significantly different between groups with an ischemic period of less than 40 min and those with more than 1 h (p<0.05), and between G-1H and G-2H (p<0.05).

Basic evaluation of myocardial $^{123}$I-MIBG uptake

Eight dogs with infarct showing $^{123}$I-MIBG defects were evaluated for myocardial noradrenaline content and were compared to $^{123}$I-MIBG uptake. When a concen ratio in ischemic areas to normal areas was compared to the noradrenaline content ratio (ischemia/normal), there was a good correlation ($r=0.867$, $y=1.07x+0.114$, p<0.05) between them. The normal $^{123}$I-MIBG uptake was different depending on the specific activity, but with the same loading dose and specific activity, absolute $^{123}$I-MIBG myocardial counts corrected by the injection dose showed a good correlation to noradrenaline contents (μg/gram muscle) ($r=0.846$) in ischemic areas.

Heart rate, blood pressure and doppler flow

The heart rate and systemic blood pressure decreased after ischemic intervention to a similar degree to the control. All through the study, including $^{123}$I-MIBG injection, the physiological condition was kept constant. Reactive hyperemia was suppressed after reperfusion and doppler flow was maintained at the control value.

Regional wall motion with time

Regional shortening with time except G-40N is shown in Fig. 1. Each group showed a statistically significant decrease during occlusion compared to the controls but with no statistical difference among the groups (during occlusion; G-20: $27±13\%$ of the control, G-40: $26±19\%$, G-1H: $18±9\%$, G-2H: $19±12\%$). However, G-20 was the only group that showed good recovery after 2 and 3 hours of reperfusion (p<0.05) compared to other groups (3h of reperfusion; G-20:...
Fig.1. Changes in regional wall motion (expressed as % of control value) with time. Four groups of dogs were compared. OCC = occlusion, CONT = control, REP = repertusion.

Fig.2. Defect area of $^{123}$I-MIBG (% of LV) and relative count (ischemic area/normal area) are shown. $^{123}$I-MIBG defect and decreased tissue counts appeared with an occlusion time of more than 40 minutes.

$113 \pm 6\%$, G-40: $59 \pm 3.0\%$, G-1H: $46 \pm 25\%$, G-2H: $45 \pm 17\%$).

$^{123}$I-MIBG uptake in the myocardium

In groups G-20 and G-40, $^{201}$TI and $^{123}$I-MIBG showed no defect in ex-vivo imaging and no decrease in tissue content in ischemic areas (Fig. 2). With 1 and 2 h of occlusion, however, $^{123}$I-MIBG uptake decreased and ex-vivo imaging as well. Therefore, $^{123}$I-MIBG was normally taken up in the areas with ischemia but without infarct or with small infarct. Areas of defect and relative counts at ischemic areas with 1 and 2 h of occlusion were $16.5 \pm 4.3\%$, $56 \pm 27\%$ in 1 h and $20.6 \pm 6.9\%$, $29.9 \pm 14.5\%$ in 2 h, respectively. A ratio of $^{123}$I-MIBG uptake to $^{201}$TI showed a poor correlation with infarct size.
Fig. 3. Comparison between regional wall motion (% of control) and $^{123}$I-MIBG counts (Ct/gram/BW). This showed a good correlation ($r=0.80$).

**Fig. 4.** Comparison of regional wall motion in the group with 40 minutes' occlusion either with noradrenaline (NA) infusion or without. NA infusion showed a remarkable recovery of wall motion and an increase in $^{123}$I-MIBG/$^{201}$TI count ratio. ISC/NS = same as Fig. 2.

determined with TTC ($r=0.54$). Thus, $^{123}$I-MIBG per myocardial flow was poorly correlated to infarct size. When $^{123}$I-MIBG uptake (Ct/gram/BW) was compared to regional wall motion (Fig. 3), there was a good correlation ($r=0.80$, $y=26.6 + 196.8x$) between them in cases with infarct. There was also a good correlation between regional wall motion and regional $^{123}$I-MIBG uptake per flow ($^{123}$I-MIBG/relative $^{201}$TI uptake in ischemic area compared with normal area) ($r=0.83$, $y=94.6 - 65.9$). Wall motion is, therefore, related not only to $^{123}$I-MIBG uptake but also to myocardial flow, but poorly related to infarct size in the acute phase of infarct with stunning.

**Effect of noradrenaline infusion for wall**
motion and $^{123}$I-MIBG uptake

In 1 h of reperfusion after 40 min of coronary occlusion, regional wall motion showed remarkable improvement after noradrenaline infusion within an hour (2 hours after reperfusion; G-40: $44.1 \pm 0.35\%$, G-40N: $142 \pm 7\%$, $p < 0.05$), although myocardial stunning continued without noradrenaline infusion (Fig. 4). At the same time $^{123}$I-MIBG uptake per unit myocardial flow showed significant increase compared to the control (G-40: $1.04 \pm 0.03$, G-40N: $1.19 \pm 0.1$, $p < 0.05$) (Fig. 4).

DISCUSSION

We have clearly shown in this paper that $^{123}$I-MIBG uptake which was closely related to myocardial noradrenaline content was within normal limits in the stunning model or with minimal myocardial infarct. From the result of noradrenaline infusion which increased $^{123}$I-MIBG uptake with improved stunning, it is suggested that quantitative effect of catecholamine plays an important role, and 1.3 to 1.4 times of normal myocardial $^{123}$I-MIBG uptake appears to be necessary to maintain good wall motion after reperfusion.

$^{123}$I-MIBG, an analog of the adrenergic-nervous-blocking agent guanethidine, has recently been used to image the heart in dogs and rhesus monkeys, and initial studies suggest that $^{123}$I-MIBG may be stored in adrenergic neurons by the same mechanism as noradrenaline. Uptake of $^{123}$I-MIBG and noradrenaline into the canine adrenal medulla was effectively blocked by pretreatment with desmethylimipramine (DMI), suggesting $^{123}$I-MIBG uptake reflects the uptake-one system. Another paper reports that the regional denervation produced by phenol application to the surface of the myocardium and stellate ganglion removal showed reduced uptake of $^{123}$I-MIBG evaluated with norepinephrine content.

It was observed that a positive inotropic intervention such as the infusion of epinephrine, postextrasystolic potentiation or exercise may result in substantial improvement in regional function, i.e. the demonstration of "contractile reserve" in the stunned myocardium. In canine myocardium, shortly after the onset of acute ische-

mia, the contractile response to sympathetic neural stimulation is ablated, while responsiveness to exogenous norepinephrine is maintained. An other paper suggested that stunning was unlikely to be simple depletion of noradrenaline from the nerve terminals in the ischemic zone, although the mechanism of the loss of response to sympathetic stimulation is unknown. Although severe ischemia is a profound mediator for release of noradrenaline, histochemical studies demonstrate only a small amount of depletion of noradrenaline from nerve terminals after up to 30 min of ischemia. The dramatic increase in segmental shortening in the postischemic segment after infusion of bretylium, however, indicated that noradrenaline was present in the nerve terminals. This report supported our results that in less than 40 min of ischemia $^{123}$I-MIBG uptake remained within normal limits, and that exogenous stimulation by infusion of noradrenaline enhanced the regional wall motion and $^{123}$I-MIBG uptake.

Thus, uptake mechanism and response to exogenous noradrenaline infusion seems to be intact with short periods of ischemia in spite of decreased regional wall motion. However, more severe ischemia with significant infarction showed impaired storage and uptake of $^{123}$I-MIBG at the nerve endings. Deterioration of wall motion was linearly related to $^{123}$I-MIBG content per flow, meaning total absence of catecholamine even after recovery of the release mechanism in 2.5–5 h of ischemia.

The reason for the depressed contraction with a capability of responding to exogenous noradrenaline, in spite of normal $^{123}$I-MIBG concentration at nerve endings, is still unknown. Acute ischemia in dogs showed no detectable changes in beta-adrenergic receptor density, but showed a significant decline in adenylate cyclase activity. Other factors, such as calcium-dependent noradrenaline release from presynaptic nerve endings, blockade of neurotransmitter release by adenosine, hydrogen ion or potassium in the ischemic myocardium, may relate to myocardial stunning.

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