Abnormal Myocardial Presynaptic Norepinephrine Recycling in Patients With Brugada Syndrome

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Background—Life-threatening ventricular tachyarrhythmias can occur in young patients without structural heart disease (idiopathic forms). In many patients, these are typically triggered by an increased sympathetic tone, eg, by physical or mental stress. In contrast, in Brugada syndrome, ventricular tachyarrhythmias more often occur during rest or sleep when the vagal tone is predominant. Furthermore, adrenergic agonists can reduce the level of ST-segment elevation, whereas it is increased by parasympathetic agonists or adrenergic antagonists. The aim of this study was to investigate presynaptic and postsynaptic myocardial sympathetic function in patients with Brugada syndrome.

Methods and Results—Nine patients with Brugada syndrome (6 male, 3 female; age, 41 ± 13 years) were enrolled in this study. The cardiac autonomic nervous system was assessed noninvasively, quantifying myocardial presynaptic and postsynaptic sympathetic function by means of positron emission tomography with the norepinephrine analogue 11C-Hydroxyephedrine (11C-HED) and the nonselective β-blocker 11C-CGP 12177 (11C-CGP). Presynaptic sympathetic norepinephrine recycling, assessed by 11C-HED, was globally increased in patients with Brugada syndrome compared with a group of age-matched healthy control subjects (92.9 ± 16.2 mL/g versus 69.1 ± 14.2 mL/g; P < 0.05), whereas postsynaptic β-adrenoceptor density, assessed by 11C-CGP, was similar in patients and control subjects (10.4 ± 6.7 pmol/g versus 10.2 ± 2.9 pmol/g; P = NS).

Conclusions—The present study on autonomic innervation in Brugada syndrome describes an enhanced presynaptic norepinephrine recycling with preserved β-adrenoceptor density, further supporting the hypothesis of an autonomic dysfunction in Brugada syndrome. This is a further step toward the understanding of the pathophysiology of the disease with potential future impact on therapeutic strategies. (Circulation. 2004;110:3017-3022.)

Key Words: fibrillation • death, sudden • nervous system, autonomic • receptors, adrenergic, beta

In 1992, Brugada et al1 described a syndrome characterized by right bundle-branch block, ST-segment elevation in the right precordial leads, and sudden cardiac death in patients without evidence of structural heart disease. This condition, known as Brugada syndrome, accounts for ≈20% to 30% of cases previously diagnosed as idiopathic ventricular fibrillation.2 The pathophysiology of this syndrome remains poorly understood. In 1998, Chen et al3 discovered the first genetic defect, a mutation in the cardiac sodium channel gene SCN5A. Although several additional mutations in the same gene have been described afterward, these defects were identified in only a minority of patients reported in the literature.4,5

Clinical observations indicate an involvement of the cardiac autonomic nervous system in the onset of ventricular tachyarrhythmias in patients with Brugada syndrome in whom ventricular tachycardia, syncope, and/or cardiac arrest occur more frequently during rest or sleep, when the vagal tone is predominant.6 Furthermore, the magnitude of ST-segment elevation can be reduced by adrenergic agonists, whereas it is increased by parasympathetic agonists or adrenergic antagonists.7,8 These latter findings are opposite to what is generally observed in patients with other forms of idiopathic ventricular tachyarrhythmias, who show a tendency to have arrhythmias during or immediately after exercise or stress, at a time when sympathetic activity is predominant.9

Using single-photon emission computed tomography (SPECT) and the norepinephrine analogue [123I]-metaiodobenzylguanidine ([123I]-MIBG), we have demonstrated a regionally reduced tracer uptake in the inferior left ventricular wall in 47% of our patients with Brugada syndrome.10 Similar results were previously obtained in patients with arrhythmio-
genic right ventricular cardiomyopathy (ARVC) and in those with right ventricular outflow tract tachycardia (RVOT).

Studies using positron emission tomography (PET) with \(^{11}C\)-hydroxyephedrine \((^{11}C\text{-HED})\) and \(^{11}C\)-CGP (Ciba-Geigy product) 12177 have shown that both ARVC and RVOT were characterized by reduced presynaptic catecholamine recycling and downregulation of postsynaptic \(\beta\)-adrenoceptors.\(^{13,14}\)

In the present study, we aimed to further investigate and prove our previous observations made with SPECT and \(^{125}\text{I}\)-MBG in patients with Brugada syndrome by noninvasive quantification of myocardial presynaptic and postsynaptic sympathetic function, using PET with \(^{11}C\text{-HED}\) and \(^{11}C\text{-CGP}\).

The results in patients were compared with those obtained in 2 groups of age-matched normal volunteers.

### Methods

**Patients**

Patients with Brugada syndrome referred to the Department of Cardiology at the University of Münster were screened. To exclude potential confounding effects of other parameters on the sympathetic nervous system, only those patients with no evidence of other cardiac conditions such as coronary artery disease were asked to take part in the study. The investigations during this screening included noninvasive (echo, stress, and rest ECGs) as well as invasive (angiogram, electrophysiological studies, biopsy) techniques.

The clinical and electrophysiological characteristics of the patients are given in the Table. Nine patients (6 male, 3 female), 41±13 years of age, were investigated. Seven patients showed typical ST-segment elevation in the baseline surface-ECG of either the coved (n=3) or the saddle-back type (n=4). The remaining 2 had an indeterminate ECG type (Table). The intravenous administration of the sodium channel blocker ajmaline (1 mg/kg) provoked an increased ST-segment elevation in the right precordial leads in all patients (Table). The intravenous administration of the sodium channel blocker ajmaline (1 mg/kg) provoked an increased ST-segment elevation in the baseline surface-ECG of either the coved (n=3) or the saddle-back type (n=4). The remaining 2 had an indeterminate ECG type (Table). The intravenous administration of the sodium channel blocker ajmaline (1 mg/kg) provoked an increased ST-segment elevation in the right precordial leads in all patients (Table).

All patients underwent programmed electrical stimulation as reported previously. In 7 patients, a cardioverter-defibrillator (ICD) was implanted. One patient refused the ICD and 1 patient received an event recorder. Only 1 patient (No. 1) had an episode of recurrent ventricular tachyarrhythmia with appropriate ICD discharge during a mean follow-up of 37 months. A mutation in the sodium channel gene SCN5A was found in 1 patient (11%). The single-stranded conformational polymorphism analysis of the entire coding regions of the cardiac sodium channel gene SCN5A was performed as reported previously.\(^{16}\)

**Control Groups**

For the studies with \(^{11}C\text{-HED}\), the control group consisted of 11 healthy volunteers (6 male, 5 female), 35±9 years of age (P=NS versus patients). For the \(^{11}C\text{-CGP}\) scans, a second group of 19 healthy volunteers (13 male, 6 female), 43±5 years of age (P=NS versus patients), was investigated. All control subjects had low-risk profiles, normal examination results, resting 12-lead ECGs, and exercise tests. No control subject was receiving drug treatment or had a history, signs, or symptoms of diseases possibly affecting the sympathetic nervous system.

All patients and control subjects gave written informed consent to the study protocol, which was approved by the Research Ethics Committee of the University Hospital Münster, the Hammersmith Hospital Research Ethics Committee, and the United Kingdom Administration of Radioactive Substances Advisory Committee.

**PET Data Acquisition and Analysis**

At the time of the PET studies, all subjects were off medication for at least 5 half-times and off smoking and caffeine-containing drinks for at least 24 hours. Investigations were performed in the nonseated resting state after fasting for at least 4 hours. All 9 patients were scanned with oxygen-15–labeled carbon monoxide \((^{15}\text{O})\) for the assessment of blood volume followed by \(^{11}C\text{-HED}\) to measure myocardial presynaptic \(^{11}C\text{-HED}\) scan could not be analyzed because of technical problems. In 5 of the 9 patients, an additional PET study was carried out the day after the \(^{11}C\text{-HED}\) scan to measure myocardial \(\beta\)-adrenoceptor density using \(^{11}C\text{-CGP}\) after acquisition of a second \(^{15}\text{O}\) scan.

The PET studies were carried out with the use of an ECAT 931 to 08/12 PET camera (Siemens/CTI) whose characteristics have been previously described.\(^{13,19}\) The left ventricle was centered in the scanner field of view by means of a rectilinear scan recorded during the exposure of external germanium-68 ring sources. This was followed by a 20-minute transmission scan for attenuation correction of all subsequent emission data. During scanning, one ECG lead was continuously monitored and blood pressure and 12-lead ECG were recorded at regular intervals.

Normalization, attenuation, correction, reconstruction, and data analysis were performed as reported previously.\(^{15,14,19}\)

**Presynaptic Norepinephrine Recycling**

\(^{11}C\text{-HED}\) was prepared and infused intravenously as described previously.\(^{15,14}\) Norepinephrine recycling was assessed by calculat-
ing the volume of distribution \( V_d \) of \(^{11}\text{C}\)-HED with the use of a single-tissue compartment model.\(^{13,14}\) The arterial input function was obtained from a left atrial region of interest on the dynamic \(^{11}\text{C}\)-HED scan for the first 15 minutes after tracer’s infusion and by means of a peristaltic withdrawal pump and a bismuth germanate (BGO) detection system afterward.\(^{19,20}\)

Plasma metabolite concentrations from additional blood samples were determined by high-performance liquid chromatography and used with the measured whole blood to plasma ratios to provide the plasma \(^{11}\text{C}\)-HED input curves. To convert \( V_d \) from units of milliliter per milliliter to milliliter per gram of tissue, all values were divided by the density of myocardial tissue (1.04 g/mL tissue).\(^{13,14}\)

**Myocardial \( \beta \)-Adrenoceptor Density**

The measurement of myocardial \( \beta \)-adrenoceptor density using \(^{11}\text{C}\)-CGP was performed according to a modification of the double-injection protocol previously reported.\(^{21,22}\) Briefly, during a dynamic emission scan, a first dose of \(^{11}\text{C}\)-CGP with high specific activity was infused intravenously over a 2-minute period. Thirty minutes later, a second dose with low specific activity was again infused over 2 minutes. The \( \beta \)-adrenoceptor density was calculated by measurement of the maximal specific binding capacity \( (B_{\text{max}}, \text{pmol/g}) \) of the \( \beta \)-adrenergic receptor antagonist \(^{11}\text{C}\)-CGP. \( B_{\text{max}} \) was calculated with the use of a modification of the equation described by Delforge et al.\(^{21}\) to take account of the molar content of \(^{11}\text{C}\)-CGP in both injections.\(^{22}\) To convert \( B_{\text{max}} \) from units of picomoles per milliliter of tissue to picomoles per gram of tissue, all values were divided by the density of myocardial tissue (1.04 g/mL tissue).

**Statistical Analysis**

Results are expressed as mean\(\pm\)SD. After testing for the equality of variances (Levene test), the Student \( t \) test for unpaired data was used to compare the global and regional values of \( V_d \) and \( B_{\text{max}} \) between groups. A value of \( P<0.05 \) was considered significant.

**Results**

**Hemodynamic Parameters**

In all patients and control subjects, heart rate and blood pressure were within normal limits at baseline and throughout the PET scans.

**Presynaptic Catecholamine Uptake**

The \( V_d \) of \(^{11}\text{C}\)-HED was globally increased in patients compared with control subjects (92.9\(\pm\)16.2 mL/g versus 69.1\(\pm\)14.2 mL/g; \( P=0.001 \); Figure 1). There were no differences in \(^{11}\text{C}\)-HED \( V_d \) among the 4 myocardial regions in the patients who all had significantly lower \( V_d \) values compared with control subjects (Figure 2A).

![Figure 1. Increased presynaptic norepinephrine recycling (Vd of \(^{11}\text{C}\)-HED, left) and preserved postsynaptic \( \beta \)-adrenoceptor density (Bmax assessed by \(^{11}\text{C}\)-CGP 12177; right) in Brugada patients compared with age-matched control subjects (individual values, mean\(\pm\)SD).](image1)

![Figure 2. A, Increased presynaptic norepinephrine recycling (Vd of \(^{11}\text{C}\)-HED, mL/g) in Brugada patients (white bars) compared with control subjects (black bars) for different parts of the left ventricle. *\( P<0.001 \). B, No difference in \( \beta \)-adrenoceptor density (Bmax of \(^{11}\text{C}\)-CGP 12177, pmol/g) between Brugada patients (white bars) and control subjects (black bars).](image2)
Myocardial \( \beta \)-Adrenoceptor Density

Myocardial \( \beta \)-adrenoceptor density (B\(_{\text{max}}\)) in patients with Brugada syndrome was comparable to normal control subjects (10.4±6.7 pmol/g versus 10.2±2.9 pmol/g; \( P=\text{NS} \); Figure 1). Only 1 patient (No. 1) showed an increased \( \beta \)-adrenoceptor density (21.9 pmol/g) compared with all other Brugada patients as well as all control subjects. Figure 2B shows the regional distribution of B\(_{\text{max}}\); it is comparable in all 4 left ventricular regions in both Brugada syndrome and control subjects.

**Discussion**

In a recent article\(^{10}\) investigating the myocardial presynaptic sympathetic innervation by means of SPECT with \(^{123}\)I-MIBG, our group described an impaired sympathetic innervation in patients with Brugada syndrome. We hypothesized that this could lead to an autonomic imbalance, potentially triggering the observed life-threatening tachyarrhythmias. Using sophisticated quantitative PET technology in the present study, we found an increased myocardial presynaptic catecholamine recycling (\( V_d \) of \(^{11}\)C-HED) in patients with Brugada syndrome despite preserved \( \beta \)-adrenoceptor density (B\(_{\text{max}}\) of \(^{11}\)C-CGP 12177), further supporting our hypothesis of an autonomic imbalance in these patients.

These findings differ from those obtained in patients with ARVC, RVO-VT, and hypertrophic cardiomyopathy (HCM), in whom both \( V_d \) of \(^{11}\)C-HED and B\(_{\text{max}}\) of \(^{11}\)C-CGP 12177 were reduced compared with control subjects. In the latter 3 conditions, the reduced myocardial \( V_d \) of \(^{11}\)C-HED probably reflects a net increase of norepinephrine concentration in the synaptic cleft, which in turn might be the cause of myocardial \( \beta \)-adrenoceptor downregulation.\(^{13,14,19}\) On the other hand, in patients with Brugada syndrome, the increased myocardial \( V_d \) of \(^{11}\)C-HED would be consistent with a reduction of norepinephrine concentration in the synaptic cleft, a hypothesis that would be in keeping with the lack of \( \beta \)-adrenoceptor downregulation.

**Clinical Evidence of Autonomic Dysfunction in Brugada Syndrome**

The findings of the present study agree with the clinical observations that patients with Brugada syndrome typically have their arrhythmic events at rest or during sleep, when there is parasympathetic dominance.\(^6\) This is also supported by ECG changes that become manifest in these patients after pharmacological modulation of the adrenergic and/or vagal tone.\(^7\) Sympathetic agonists and parasympathetic antagonists diminish right precordial ST-segment elevation, whereas adrenergic blockers and parasympathetic agonists aggravate the typical ECG signs of Brugada syndrome. Interestingly, the parasympathetic transmitter acetylcholine is known to affect ion currents such as \( I_a \) and \( I_{Ca} \).\(^{23}\) These are more prominent in the epicardium than in the endocardium, explaining the so-called spike-and-dome morphology of the action potential. Autonomic imbalance with reduced adrenergic nerve activity and dominant vagal tone may therefore modulate epicardial ion currents, resulting in a loss of the action potential dome with subsequent elevation of the ST segment in the right precordial surface ECG. This mechanism may theoretically lead to increased transmural dispersion of refractoriness and subsequently to a higher propensity for the onset of ventricular tachyarrhythmias. Although this hypothetical concept is not yet proved, it would be in line with the clinical observation that ECG signs of Brugada syndrome were most prominent immediately before or after an arrhythmic event, strongly suggesting that the extent of ST-segment elevation correlates with the risk of occurrence of arrhythmias.\(^{24,25}\) Recent studies reported that symptomatic patients with manifest ECG signs at baseline are at higher risk compared with asymptomatic patients with ECG signs present only after provocative test with intravenous sodium channel blockers. A coved-type ST-segment elevation appears to be associated with a higher risk when compared with a saddle-back configuration.\(^{24,25}\) The extent of ECG abnormalities therefore appears to correlate with the risk of arrhythmic events. In consequence, an autonomic imbalance that aggravates these ECG signs may be a relevant cofactor for arrhythmia occurrence and prognosis in Brugada syndrome.

**Mechanisms of Autonomic Dysfunction in Brugada Syndrome**

Our results demonstrate an increase of the volume of distribution (\( V_d \)) of the norepinephrine analogue \(^{11}\)C-HED in patients with Brugada syndrome [(1) in Figure 3]. Because \( V_d \) integrates neuronal influx (uptake-1) and efflux (release) of \(^{11}\)C-HED, increased \( V_d \) in patients with Brugada syndrome may result from either enhanced uptake-1 activity or diminished release of norepinephrine into the synaptic cleft. Both mechanisms would result in reduced levels of norepinephrine in the synaptic cleft [(2) in Figure 3]. In theory, this should trigger a postsynaptic response, such as upregulation of postsynaptic \( \beta \)-adrenoceptors or changes of the intracellular signal transduction cascade (eg, G-protein–related receptor kinases) [(3) in Figure 3]. Downregulation of \( \beta \)-adrenoceptors has been demonstrated in a variety of cardiac diseases, particularly in heart failure,\(^{26}\) whereas an upregulation above normal levels has been described in transgenic mouse models\(^{27,28}\) but not yet found in any cardiac disease in humans. Although the subgroup of patients in which the \( \beta \)-adrenoceptor density was investigated is small and the data therefore appear preliminary, our finding of normal \( \beta \)-adrenoceptor densities in the majority of these patients is in line with these previous observations. However, a reduced synaptic norepinephrine concentration would even in a case of unchanged \( \beta \)-adrenoceptor density result in decreased intracellular cAMP levels, which are known to be arrhythmogenic.\(^{28}\)

Only 1 single patient (No. 1) showed a \( \beta \)-adrenoceptor density above the levels measured in the control group. Figure 4 shows the normal \(^{11}\)C-CGP images and time-activity curve of the whole heart for this patient, indicating that the measured values are not due to a faulty analysis.

**\(^{123}\)I-MIBG-SPECT Versus \(^{11}\)C-HED-PET**

In our previous study using \(^{123}\)I-MIBG and SPECT, we found a regionally reduced \(^{123}\)I-MIBG uptake mainly located in the inferior and inferoseptal wall. In the present study, using
quantitative $^{11}$C-HED-PET, this finding of an impaired pre-synaptic sympathetic innervation was further supported by the observation of a globally increased norepinephrine recycling. However, regional differences of HED accumulation did not reach statistical significance.

Acquisition techniques (SPECT versus PET) for the 2 tracers are different. Although the regional distribution of $^{123}$I-MIBG is measured by static SPECT 4 hours after injection, $^{11}$C-HED is measured by dynamic PET over a time period of 1 hour after injection. Therefore, regional differences in myocardial tracer accumulation resulting from a differential regional washout of the radioactively labeled catecholamine analogue can be missed by the PET protocol used in the present study, whereas the late SPECT image can detect these. Furthermore, in contrast to $^{11}$C-HED, the uptake of $^{123}$I-MIBG is known to be reduced in the inferior/inferoseptal wall in cases of increased parasympathetic tone, resulting from the more pronounced parasympathetic innervation of the inferior left ventricular wall and the right ventricle when compared with the remaining myocardium.

Therefore, a reduced $^{123}$I-MIBG uptake in these areas can result from a regionally diminished presynaptic sympathetic noradrenaline recycling or, as in the case of Brugada syndrome, from a globally increased presynaptic sympathetic noradrenaline recycling with a regional mismatch between parasympathetic and sympathetic tone in the inferior wall. This autonomic imbalance due to a reduced sympathetic activity (subsequent to a reduced presynaptic neurotransmitter release or an enhanced norepinephrine reuptake, both resulting in lower norepinephrine levels in the synaptic cleft) has already been proposed to be the most likely explanation for the regional $^{123}$I-MIBG defects in the previous report.

Therefore, the present quantitative PET data with an increased uptake-1 resulting in reduced synaptic levels of norepinephrine support our previous hypothesis.
Interestingly, the $^{123}\text{I}-\text{MIBG}$ scans in our study population showed a reduced uptake in the inferior wall only in those 3 patients with the highest values of $V_d$ of $^{11}\text{C}-\text{HED}$ ($>100$ mL/g).

**Limitations of the Study**

All patients enrolled in this study are well characterized and selected according to detailed noninvasive and invasive investigations. Although we screened all patients with Brugada syndrome referred to the Department of Cardiology and Angiology at the University of Münster, only a small subgroup fulfilled the entry criteria for the present study. Therefore, the number of patients studied is small. In particular, measurement of myocardial $\beta$-adrenoceptor density was obtained only in 5 of the 9 patients. However, Brugada syndrome is a rare disease, and quantitative PET assessment of both presynaptic and postsynaptic myocardial innervation is a complex investigation, presently only available at the MRC Clinical Sciences Centre in London.

The parasympathetic branch of the autonomic nervous system was not investigated in the present study. Therefore, conclusions on the vagal innervation remain hypothetical and quantitative assessments of the parasympathetic innervation as well as investigations on the cellular signal transduction are relevant future steps toward a more complete understanding of the pathophysiology of Brugada syndrome.

**Conclusions**

The present study on autonomic innervation in Brugada syndrome describes for the first time an enhanced presynaptic norepinephrine recycling with preserved $\beta$-adrenoceptor density. This is a novel finding in Brugada syndrome and a further step toward the understanding of the pathophysiology of the disease. Today, the implantable cardioverter/defibrillator is the leading therapeutic option for patients with Brugada syndrome. The findings of the present study in combination with future investigations of the parasympathetic branch of the autonomic nervous system may potentially lead to the development of target-specific therapeutic strategies in Brugada syndrome.

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