Up-Regulation of Inositol 1,4,5 Trisphosphate Receptor Expression in Atrial Tissue in Patients With Chronic Atrial Fibrillation

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OBJECTIVES
We examined whether patients with atrial fibrillation (AF) have alterations in atrial inositol 1,4,5 trisphosphate receptors (IP3 receptors).

BACKGROUND
Abnormal intracellular Ca\(^{2+}\) homeostasis occurs in chronic AF. The intracellular Ca\(^{2+}\) concentration is regulated by ryanodine and IP3 receptors. We recently reported alterations in ryanodine receptors in atrial tissue from patients in chronic AF.

METHODS
We analyzed IP3 receptor expression in the right atrial myocardium from 13 patients with mitral valvular disease (MVD) with AF (MVD/AF), five patients with MVD who had normal sinus rhythm (MVD/NSR) and eight control patients with NSR (tissue obtained during coronary artery bypass surgery). Hemodynamic and echocardiographic data were obtained preoperatively, and an immunohistochemical study was performed on atrial tissue.

RESULTS
The relative expression level of IP3 receptor protein was significantly greater in MVD/AF (0.75 ± 0.26) than it was in MVD/NSR (0.42 ± 0.13, p < 0.01), and both were significantly above control (0.14 ± 0.08). The relative expression level of IP3 receptor messenger RNA was significantly greater in the MVD/AF group (0.028 ± 0.008) than it was in the control group (0.015 ± 0.004, p < 0.01), but patients with MVD/AF did not differ from patients with MVD/NSR (0.020 ± 0.006). The relative expression levels of IP3 receptor protein and messenger RNA were higher in patients with left atrial dimension ≥40 mm, pulmonary capillary wedge pressure ≥10 mm Hg and right atrial pressure ≥5 mm Hg. Inositol 1,4,5 trisphosphate receptors were over-expressed in the cytosol and at the nuclear envelope of atrial myocytes in MVD.

CONCLUSIONS
Since chronic mechanical overload of the atrial myocardium increased IP3 receptor expression, especially in patients with chronic AF, up-regulation of IP3 receptors may be important in modulating intracellular Ca\(^{2+}\) homeostasis and initiating or perpetuating AF. (J Am Coll Cardiol 2001;37:1111–9) © 2001 by the American College of Cardiology

Atrial fibrillation (AF) is the most frequently encountered arrhythmia in the clinical setting. The main electrophysiological mechanism underlying AF appears to be a progressive decrease in atrial refractoriness, a phenomenon that has been termed “electrical remodeling” (1). Although this phenomenon is thought to be of importance in the self-perpetuation of AF and although cytosolic Ca\(^{2+}\) abnormalities are an important mediator of sustained AF, the cellular and molecular mechanisms initiating or maintaining AF remain unclear. Recently, we reported that in the atrial tissue of patients with chronic AF, there are alterations in ryanodine receptors (sarcoplasmic reticulum [SR] Ca\(^{2+}\) regulatory proteins), which play an important role in regulating the intracellular Ca\(^{2+}\) concentration in cardiac muscle (2).

Two forms of intracellular Ca\(^{2+}\) release channels serve to regulate the intracellular Ca\(^{2+}\) concentration: the ryanodine receptor (RyR) and the inositol 1,4,5 trisphosphate (IP3) receptor. Recently, an IP3 receptor was detected in both intracellular and plasma membrane fractions of canine pancreatic homogenates (3) and also in cardiac myocytes (4), in which it was restricted to the region of the intercalated discs (5). The demonstration that both forms of Ca\(^{2+}\) release channels are expressed in cardiac myocytes raises the possibility that a complex feedback mechanism may exist involving interactions between IP3 receptor and RyR Ca\(^{2+}\) pools. Moreover, Kijima et al. (5) suggested that a possible role of the cardiac IP3 receptor may be to mediate Ca\(^{2+}\) entry or to modulate intercellular communication via junctions.

The purpose of this study was to determine whether patients with chronic AF have alterations in the IP3 receptor in their atrial tissue. We studied the expression levels of IP3 receptor protein and messenger RNA (mRNA) in the right atrium after its removal during cardiac surgery from patients with chronic AF and from others with a normal sinus rhythm (NSR).

METHODS
Selection of patients. We examined right atrial tissue (atrial appendage) from: 1) 13 patients with mitral valvular...
disease (MVD) in whom AF had been sustained for more than two years, and 2) 5 patients with MVD who had NSR; tissue was obtained during cardiac surgery for MVD in all 18 patients. Control material (right atrial tissue from eight patients without MVD who had NSR) was obtained during coronary artery bypass surgery. All patients were evaluated by the Second Department of Internal Medicine, Yamaguchi University School of Medicine, and they underwent surgery at the Yamaguchi University Hospital. Informed consent was obtained from each patient. The protocols were in accord with guidelines laid down by the Institutional Review Board, Yamaguchi University Hospital. Demographic data (age, gender, diagnosis and hemodynamic data) are shown in Table 1. In addition to their usual medication, all patients received preoperative sedation and perioperative anesthesia. Hemodynamic and echocardiographic data were obtained by reviewing data from preoperative cardiac catheterizations and echocardiograms.

**Abbreviations and Acronyms**

AF = atrial fibrillation
cDNA = complementary DNA
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
IP3 receptor = inositol 1,4,5 trisphosphate receptor
LAD = left atrial diameter
mRNA = messenger ribonucleic acid
MVD = mitral valvular disease
NSR = normal sinus rhythm
PCR = polymerase chain reaction
PCWP = pulmonary capillary wedge pressure
RAP = right atrial pressure
RT-PCR = reverse transcription-polymerase chain reaction
RyR = ryanodine receptor
SR = sarcoplasmic reticulum

**Myocardial tissue samples.** Right atrial appendages were frozen immediately and stored at $-80^\circ$C until needed. They were used to prepare a membrane fraction for a Western blotting assay and to produce RNA for reverse transcription polymerase chain reaction (RT-PCR) amplification.

**Table 1. Clinical Characteristics of Patients**

<table>
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<th>Pt. (No.)</th>
<th>Diagnosis</th>
<th>Age/Gender</th>
<th>EF (%)</th>
<th>RAP (mm Hg)</th>
<th>PCWP (mm Hg)</th>
<th>LAD (mm)</th>
<th>AF Duration</th>
<th>Presurgical Medication</th>
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<td>9</td>
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<td>90</td>
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<td>12</td>
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<td>15 ± 5†</td>
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<td>4 ± 2</td>
<td>6 ± 2</td>
<td>35 ± 4</td>
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*p < 0.05 compared with control; †p < 0.01 compared with control; ‡p < 0.05 compared with MVD + NSR.

AF = atrial fibrillation; EF = left ventricular ejection fraction; IHD = ischemic heart disease; ISDN = isosorbide dinitrate; ISMN = isosorbide mononitrate; LAD = left atrial diameter; MVD = mitral valvular disease; NSR = normal sinus rhythm; NTG = nitroglycerin; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure.
Atrial membrane preparation. A membrane preparation was obtained by differential centrifugation as described by Volpe et al. (6) with some modifications. Atrial tissue (approximately 100 mg) was homogenized using a Polytron (Kinematica, Lucerne, Switzerland) in 30 mM Tris-maleate containing 0.3 M sucrose, 5 mg/l leupeptin and 0.1 mM phenylmethylsulfonyl fluoride, pH 7.0 (solution A). The homogenate was centrifuged at 5,500 × g for 10 min, and the supernatant was centrifuged at 12,000 × g for 20 min. The supernatant was incubated for 60 min in a buffer of the following composition: 30 mM Tris-maleate, 0.3 M sucrose, 0.6 M KCl, 5 mg/l leupeptin, 0.1 M PMSF, pH 7.0, and centrifuged at 143,000 × g for 30 min. The pellet was resuspended in solution A. This fraction was rapidly frozen in liquid nitrogen and stored at −80°C. An aliquot was retained for protein assay using the method of Lowry et al. (7) with bovine serum albumin standards.

Immunologic quantification of IP3 receptor protein. Immunoblot analysis was performed as previously described (5) with some modifications. Atrial membrane protein (40 μg protein/lane) was electrophoresed on 4% SDS-polyacrylamide gels using a Laemmli buffer system (8). The proteins in the gel were transferred to a protran nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The membranes were then treated with 5% nonfat dry milk in phosphate-buffer, incubated with a polyclonal anti-IP3 receptor type-1 antibody (Affinity Bioreagents, Inc., Golden, CO) (1:1000 dilution) solution and incubated further with peroxidase-conjugated secondary antibody (1:1000 dilution).

The amount of protein recognized by the antibodies was quantified by means of an ECL™ immunoblotting detection system (Amersham Pharmacia Biotech, Buckinghamshire, England), with the membrane being exposed to X-ray film. Quantitative densitometry of immunoblots was performed using a microcomputer-imaging-device (AE-6900M, Atto, Tokyo, Japan). The relative activity associated with cardiac IP3 receptor protein in each sample was quantified using a microcomputer-imaging system (Amersham Pharmacia Biotech, Buckinghamshire, England) with the membrane being exposed to X-ray film. Quantitative densitometry of immunoblots was performed by Sanger’s method (12) using a DNA Sequencer ABI PRISM 377 XL (PE Applied Biosystem, Foster City, California). Nucleotide sequencing was performed by the method of Sanger et al. (13) using an ABI PRISM 377 XL DNA Sequencer ABI PRISM 377 XL (PE Applied Biosystem, Foster City, California).
enzyme of the glycolytic pathway is constitutively expressed in most tissues and is the most widely accepted internal control in the molecular biology literature (13).

**Light microscope immunohistochemistry.** For immunostaining of the IP3 receptor, frozen tissue was allowed to equilibrate to cryostat temperature (−30°C) and was embedded in O.C.T. Compound (Sakura Finetechical Co. Ltd., Tokyo, Japan). Then, 4 μm sections were cut, mounted on slides and dried at room temperature for 6 h. Immunostaining was performed using the avidin-biotinylated peroxidase complex. The sections were pretreated with 3% H2O2 in absolute methanol to quench endogenous peroxidase activity. Endogenous avidin and biotin were blocked according to the manufacturer’s recommendations (Dako LSAB Kit, Dako Co., Carpinteria, California). After equilibration in phosphate-buffered saline, the sections were blocked with 5% bovine serum albumin. Anti-IP3 receptor antibody (Affinity Bioreagents, Inc., Golden, CO) (1:50) was applied to alternate serial sections, and these were then incubated for 12 h at room temperature. After washing, the sections were incubated in biotinylated goat anti-rabbit IgG for 1 h. After three more washes, the sections were incubated with the avidin–biotin complex from a standard peroxidase kit (Dako LSAB Kit, Dako Co., Carpinteria, California).

**Statistical analysis.** All data are presented as mean ± SD. Comparisons between data were made by one-way analysis of variance followed by Fisher’s exact test. Differences were taken to be significant at p < 0.05.

**RESULTS**

**Clinical characteristics and hemodynamic data.** The preoperative hemodynamic, echocardiographic and clinical data for the three groups are shown in Table 1. None of the patients with MVD who had NSR nor any of the control patients had a documented history of AF. Patient number 6 in the MVD + AF group had a VVI type pacemaker (Medtronic Inc., Minneapolis, Minnesota) for one year before the operation. The values for left ventricular ejection fraction were within the normal range in all three groups. Although right atrial pressure (RAP) tended to be higher in patients with MVD who had AF than they were in patients with MVD who had NSR or in control patients, there was no statistically significant difference among the groups. However, pulmonary capillary wedge pressure (PCWP) and left atrial diameter (LAD) were both larger in patients with MVD who had either AF or NSR than they were in control patients. In patients with MVD who had AF, LAD was significantly larger than in patients with MVD who had NSR.

**Analysis of IP3 receptor expression by the Western blot method.** Figure 1 shows the expression level of the IP3 receptor protein in right atrial tissue from patients with MVD who had either AF or NSR and from control patients. The level was significantly higher in patients with MVD who had AF (0.75 ± 0.26) than it was in patients with MVD who had NSR (0.42 ± 0.13), and both values were significantly higher than that obtained for the control group (0.14 ± 0.08).
Next, the data for the expression level of IP3 receptor protein in the right atrium were pooled for all 26 patients and broken down according to the patient's LAD, PCWP or RAP. As shown in Figure 2: i) the relative expression level was 0.66 ± 0.28 in those patients with a larger LAD (≥40 mm) and 0.14 ± 0.08 in those with a smaller LAD (<40 mm), and there was a significant difference between these two groups. For PCWP (ii in Fig. 2), the relative expression level was 0.73 ± 0.26 in those with a higher PCWP (≥10 mm Hg) and 0.23 ± 0.16 in those with a lower PCWP (<10 mm Hg), the difference being significant. The relative expression level of IP3 receptor protein in patients with a higher RAP (≥5 mm Hg) (0.67 ± 0.35) was greater than it was in those with a lower RAP (<5 mm Hg) (0.32 ± 0.21) (iii in Fig. 2).

**Analysis of expression levels of the mRNA for the IP3 receptor.** We next examined the expression levels of the mRNAs encoding the IP3 receptor using total RNA extracted from the patient's atrial tissues. Figure 3 shows these levels for patients with MVD who had AF or NSR and for control patients. The expression level was significantly higher in homogenates prepared from the right atrium of patients with MVD who had AF (0.028 ± 0.008) than it was in the control group (0.015 ± 0.004). There was no significant difference in this expression level between patients with MVD who had AF and patients with MVD who had NSR (0.020 ± 0.006).

When data from all 26 patients (patients with MVD who had AF or NSR and controls) were analyzed together, a positive correlation (r = 0.681) was found between the expression level of IP3 receptor protein and that of its mRNA (Fig. 4).

Next, the data for the expression levels of IP3 receptor mRNA in the right atrium were pooled for all 26 patients and broken down according to the patient's LAD, PCWP or RAP. As shown in Figure 2: i) the relative expression level was 0.66 ± 0.28 in those patients with a larger LAD (≥40 mm) and 0.14 ± 0.08 in those with a smaller LAD (<40 mm), and there was a significant difference between these two groups. For PCWP (ii in Fig. 2), the relative expression level was 0.73 ± 0.26 in those with a higher PCWP (≥10 mm Hg) and 0.23 ± 0.16 in those with a lower PCWP (<10 mm Hg), the difference being significant. The relative expression level of IP3 receptor protein in patients with a higher RAP (≥5 mm Hg) (0.67 ± 0.35) was greater than it was in those with a lower RAP (<5 mm Hg) (0.32 ± 0.21) (iii in Fig. 2).

**Analysis of expression levels of the mRNA for the IP3 receptor.** We next examined the expression levels of the mRNAs encoding the IP3 receptor using total RNA extracted from the patient's atrial tissues. Figure 3 shows these levels for patients with MVD who had AF or NSR and for control patients. The expression level was significantly higher in homogenates prepared from the right atrium of patients with MVD who had AF (0.028 ± 0.008) than it was in the control group (0.015 ± 0.004). There was no significant difference in this expression level between patients with MVD who had AF and patients with MVD who had NSR (0.020 ± 0.006).

When data from all 26 patients (patients with MVD who had AF or NSR and controls) were analyzed together, a positive correlation (r = 0.681) was found between the expression level of IP3 receptor protein and that of its mRNA (Fig. 4).
or RAP. Figure 5 (i) shows that the relative expression level of IP3 receptor mRNA was 0.025 ± 0.007 in those patients with a larger LAD (>40 mm) and 0.015 ± 0.004 in those with a smaller LAD (<40 mm) and that there was a significant difference between these two groups. For PCWP (ii in Fig. 5), the corresponding values were 0.025 ± 0.006 in those with a higher PCWP (>10 mm Hg) and 0.018 ± 0.007 in those with a lower PCWP (<10 mm Hg), the difference being significant. The relative expression level tended to be greater in those with a higher RAP (>5 mm Hg) (0.024 ± 0.008) than it was in those with a lower RAP (<5 mm Hg) (0.020 ± 0.007), but the difference did not reach significance (iii in Fig. 5).

Immunohistochemistry by light microscopy. Figure 6 shows the immunolocalization of the IP3 receptor at the light microscopic level in right atrial tissues from representative patients. Immunoreactivity for the IP3 receptor was found both within the cytosol and at the nuclear envelope of cardiac myocytes. In patients with MVD, IP3 receptor expression was greater than it was in the control both in the cytosol and at the nuclear envelope.

Figure 4. The relation between the expression levels of inositol 1,4,5 trisphosphate receptor (IP3 receptor) protein and messenger RNA (mRNA). There was a positive correlation between the expression levels of protein and mRNA. Data are included for all 26 patients studied.

Figure 5. Relation between the expression level of inositol 1,4,5 trisphosphate receptor (IP3 receptor) messenger RNA (mRNA) in the right atrium of all patients and the magnitude of left atrial dimension (LAD) (i), pulmonary capillary wedge pressure (PCWP) (ii) and right atrial pressure (RAP) (iii). The expression level was significantly greater in those patients with a higher LAD or PCWP. The expression level tended to be greater, though not significantly, in patients with a higher RAP. Data are mean ± SD. Compare these results with those for IP3 receptor protein in Figure 2.
DISCUSSION

The main findings of this study were twofold. First, in patients with chronic AF due to MVD, there were significant increases in the expression levels of IP3 receptor protein and mRNA in the right atrium, and the expression of the IP3 receptor was increased (compared with that of patients in the control group) both in the cytosol and at the nuclear envelope of atrial myocytes. Second, in all patients:

1) there was a significant positive correlation between the expression levels of IP3 receptor protein and its mRNA, and
2) the expression levels of IP3 receptor protein and mRNA in the right atrium were greater at higher levels of left atrial dimension or PCWP (although these expression levels were not significantly affected by the level of RAP). To our knowledge, although some investigators (6,14,15) have suggested that changes in IP3/Ca\(^{2+}\) signaling might be important in cardiac arrhythmogenesis, this is the first investigation in which an up-regulation of IP3 receptor protein and mRNA levels have been detected in the atrium of patients with chronic AF as compared with patients with NSR. In contrast, other Ca\(^{2+}\) regulatory proteins (RyR) are down-regulated (2). The alterations we have detected in RyR (2) and IP3 receptors might result in the establishment of an abnormal cytoplasmic Ca\(^{2+}\) concentration and, as a consequence, initiate or perpetuate changes in the electrophysiologic properties of atrial tissue that, in turn, initiate or perpetuate AF.

Up-regulation of IP3 receptor in patients with MVD who have chronic AF. In addition to the RyR, a second type of Ca\(^{2+}\) release channel, the IP3 receptor, was identified on the endoplasmic reticulum of rodent brain and smooth muscle cells (16,17). A few years later, the IP3 receptor expressed in cardiac myocytes was found to be structurally very similar to the type 1 IP3 receptor expressed in vascular smooth muscle and the cerebellum (4). The dominant mechanism underlying excitation-contraction coupling in cardiac muscle is Ca\(^{2+}\)-induced Ca\(^{2+}\) release via the RyR (14,18), and the properties of the IP3 receptor seem to be quite different from those of the Ca\(^{2+}\) gated Ca\(^{2+}\) release channel (RyR). Go et al. (15) showed that RyR and IP3 receptors were regulated in opposite directions in failing human hearts, the RyR mRNA levels being decreased while the IP3 receptor mRNA levels were increased. Recently, we reported a decrease in SR Ca\(^{2+}\) release function, as well as a decrease in the number of RyR, during the development of volume-overloaded heart failure (19), and we also showed that there were significant decreases in the number of RyR and the expression level of RyR mRNA in patients with chronic AF due to MVD (2). The important finding of our studies is that not only is there this decrease in SR Ca\(^{2+}\) release function, but also a decrease in the number of RyR, during the development of volume-overloaded heart failure (19), and we also showed that there were significant decreases in the number of RyR and the expression level of RyR mRNA in patients with chronic AF due to MVD (2). The important finding of our studies is that not only is there this down-regulation of RyR receptor protein and mRNA levels in patients with chronic AF due to MVD, but there is also an increase in the expression of the IP3 receptor. These results are consistent with the observations of Go et al. (15) on the failing human myocardium (see preceding text). Possibly, mechanical overload of the atrial myocardium might initially cause a down-regulation of RyR, and the alternative pathway for the regulation of intracellular Ca\(^{2+}\) (via the IP3 receptor Ca\(^{2+}\) release channel) might then undergo an up-regulation and, thus, serve as a significant compensatory mechanism.

Interestingly, in the patients with MVD examined in this study, an increased immunoreactivity for the IP3 receptor was found at the nuclear envelope as well as in intracellular...
membranes. In fact, Humbert et al. (20) previously reported the existence of the IP3 receptor at the nuclear membrane, and IP3 has been shown to trigger an increase in nucleoplasmic Ca$^{2+}$ (21,22). Ca$^{2+}$ has been shown to be involved in the regulation of such typical nuclear functions as gene expression and DNA breakdown, repair and replication (23). In patients with MVD, mechanical overload of the myocardium might cause an over-expression of IP3 receptors in the nuclear envelope as a way of controlling the nucleoplasmic Ca$^{2+}$ concentration (thus regulating cellular biological functions, metabolism or hypertrophic reactions). If so, intracellular Ca$^{2+}$ release, via the IP3 receptor, might be a critical step in the control of cellular metabolism in such patients.

**Up-regulation of the IP3 receptor and its possible relation to the mechanisms causing chronic AF.** In addition to regulating contractility and facilitating cellular metabolism in the diseased myocardium, the IP3/Ca$^{2+}$ signaling pathway may play a pathological role in cardiac arrhythmogenesis (14). Gorza et al. (24) suggested that Ca$^{2+}$ release via IP3 receptors may be causally related both to an increase in automaticity and the generation of some arrhythmias. They also reported an increased accumulation of IP3 receptors in atrial myocytes in the senescent heart, and they speculated that an increased expression of IP3 receptors in atrial myocytes may play a pivotal role in the regulation of chronotropism (24). In cardiac muscle, the development of a high myoplastic Ca$^{2+}$ level during diastole (> 500 nM) due to intracellular Ca$^{2+}$ oscillations appears to induce an abnormal transient inward current (25,26). Further, data from skinned cardiac cells suggest that IP3 may only play a pathological role in cardiac arrhythmogenesis by enhancing spontaneous Ca$^{2+}$ oscillations (27). In our study, the expression levels of IP3 receptor protein and its mRNA were increased in the right atrium of patients with MVD (perhaps by way of compensation for down-regulation of the RyR receptor), and, moreover, these expression levels were higher in patients with MVD who had AF than they were in patients with MVD who had NSR. Conceivably, the increased IP3 receptor expression in the atrium of patients with MVD may be causally related to the tendency for AF to be initiated or perpetuated in such patients.

It is well known that most tachyarrhythmias begin with an early premature beat. After the wave front spreads from the premature site, the spatially inhomogeneous properties of the cellular network alter the excitation wave sufficiently to create an asymmetry of conduction, and circus movement reentry ensues (28). Recent electrophysiologic and clinical evidence suggests an expanded picture that points to an adaptive structural mechanism—the distribution of gap junctions associated with the development of microfibrosis (28)—as the major cause of most forms of AF. Moreover, a disturbance of side-to-side electrical coupling between cells (nonuniform anisotropy) is thought to play a central role in the genesis of AF (29). Sneyd et al. (30) postulated the following mechanism for intercellular wave propagation. Mechanical stimulation of a single cell initiates the production of IP3 in that cell and a consequent release of Ca$^{2+}$. Some of this IP3 moves through gap junctions to neighboring cells, releasing Ca$^{2+}$ from their internal stores. A small amount of IP3 can stimulate a large release of Ca$^{2+}$ via a positive-feedback process (whereby Ca$^{2+}$ stimulates its own release through the IP3 receptor). The sequential movement of IP3 through ever more distal cells results in a corresponding intercellular Ca$^{2+}$ wave. Interestingly, the IP3 receptor has been reported to be present in ventricular and atrial myocytes and localized to the region of the gap junction, and the gap junction serves an important role in intercellular electrical coupling so that heart muscle is electrically synchronized (5). In our study we looked for evidence of an abnormal expression of IP3 receptors in the gap junction area, but we could find no such evidence. However, if others or we can find an abnormal dispersion of IP3 receptors around gap junctions in patients with AF, it will be suggestive evidence linking IP3 receptor up-regulation causally with the conduction disturbances that initiate or perpetuate reentrant arrhythmias.

**Study limitations.** In our study, we enrolled as control patients with ischemic heart disease, but with NSR. However, they were not completely “normal,” and their right atrium might have been affected. Nevertheless, the hemodynamic data obtained by reviewing the results of preoperative cardiac catheterization and echocardiography were normal, and these control patients did not exhibit signs of right atrial overload.

An additional limitation was that we could not perform detailed histopathological and electrophysiological examinations of right atrial tissues because of the difficulty of performing studies on human tissues, and the size of the myocardial tissue sample that could be obtained from the patients during surgery was critically limited. We have speculated that the increase of IP3 receptors may cause changes in cellular excitability, but we did not directly show the evidence that the increase of IP3 receptors has “functional” consequences on cardiac electrophysiology. Clearly, an examination of electrophysiology in human atrial myocytes from patients with chronic AF would be of great interest. Although we could not carry out an electrophysiological study on small samples, the changes of SR Ca$^{2+}$ regulatory proteins could, at least in part, contribute to the maintenance of AF.

**Conclusions.** Several investigators have discussed intracellular Ca$^{2+}$ abnormality as a possible initiator or perpetuator of AF. However, there has been a lack of direct evidence of abnormalities in the modulators of intracellular Ca$^{2+}$ homeostasis. In this study, we report that chronic mechanical overload of the atrial myocardium is associated with an increase in the expression level of the IP3 receptor and that this level was greater in patients with chronic AF. These results suggest that, in patients with chronic AF, up-regulation of IP3 receptors may be important in modulating...
intracellular Ca\(^{2+}\) homeostasis and, possibly, in the initiation or perpetuation of AF.

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