Valsartan Restores Sarcoplasmic Reticulum Function With No Appreciable Effect on Resting Cardiac Function in Pacing-Induced Heart Failure

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Background—Although angiotensin II receptor blockade is considered to be useful for the treatment of human heart failure, little beneficial hemodynamic effect has been shown in some experimental failing hearts. In this study, we assessed the effect of an angiotensin II receptor blocker, valsartan, on sarcoplasmic reticulum (SR) function, definitiveness of which is a major pathogenic mechanism in heart failure.

Methods and Results—SR vesicles were isolated from dog left ventricular muscle (normal or exposed to 4-week rapid ventricular pacing with or without valsartan). In the untreated and valsartan-treated paced dogs, cardiac function showed similar deterioration (compared with before pacing). However, both the density of β-receptors and the contractile response to dobutamine were greater in the valsartan-treated paced dogs than in the untreated paced dogs. In untreated paced hearts, the ryanodine receptor was protein kinase A–leak from RyR.5

Conclusions—During the development of pacing-induced heart failure, valsartan preserved the density of β-receptors and concurrently restored SR function without improving resting cardiac function. (Circulation. 2004;109:911-919.)

Key Words: sarcoplasmic reticulum  ■  heart failure  ■  calcium  ■  ion channels

In cardiac muscle, the sarcoplasmic reticulum (SR) plays an important role in excitation-contraction coupling through the regulation of the intracellular free Ca2+ concentration. An altered function of the Ca2+-release channel of the sarcoplasmic reticulum (ryanodine receptor [RyR]) has been shown to contribute to cardiac dysfunction in heart failure.2 We previously reported that in a dog model of pacing-induced heart failure, a prominent abnormal Ca2+-leak occurs through the RyR owing to a partial loss of RyR-bound FKBP12.6.3 β-Adrenergic receptor blockade has been shown to correct the defective interaction of FKBP12.6 with RyR4,5 that is triggered by the protein kinase A (PKA)-mediated hyperphosphorylation of RyR.6 Indeed, this correction leads to a restoration of the stoichiometry of the RyR2 macromolecular complex,4 a normalization of the single-channel function of RyR,4 and a prevention of the Ca2+-leak from RyR.5 Recently, we found that a new cardioprotective agent, JTV519, corrects the defective FKBP12.6-mediated stabilization of RyR, leading to an improvement in cardiac function during the development of heart failure.7 These studies strongly suggest that improving the FKBP12.6-mediated stabilization of RyR could be a new therapeutic strategy against heart failure.

Angiotensin II antagonism leads to an attenuation of the downregulation of Ca2+-ATPase (SERCA) and to an improvement in intracellular Ca2+ handling.8,9 The corrections in SR function attributable to angiotensin II antagonism may be partly responsible for the favorable effects of angiotensin II antagonism on contractile and relaxation functions. However, it is not known whether renin-angiotensin II antagonism can correct the defective interaction of FKBP12.6 and RyR that occurs in the SR during the development of heart failure. In this study, we used a dog model to assess whether chronic administration of valsartan can both correct SR function and have a beneficial effect on left ventricular (LV) function and remodeling during the development of heart failure.

Methods
FK506 and valsartan were provided by Fujisawa Pharmaceutical (Osaka, Japan) and Novartis Pharmaceutical (Tokyo, Japan), respectively.

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Determination of the Dose of Valsartan for Chronic Administration

To determine the dose of valsartan for chronic administration, we evaluated the concentration-dependent effect of valsartan on hemodynamic parameters in normal conscious dogs. Valsartan (0.1 to 1 mg/kg per d) was orally administered for 3 days/each dose, starting at a dose of 0.1 mg/kg per d and increasing incrementally to 1 mg/kg per d. Valsartan (≥0.5 mg/kg per d) was found significantly to decrease both LV pressure and the peak +dP/dt of LV pressure. We then decided to try a much lower dose (0.1 mg/kg per d) for chronic administration in the following experiments to eliminate the blood pressure–lowering effect of valsartan. At this dose, neither peak systolic pressure nor the baseline peak +dP/dt was decreased. Moreover, when angiotensin II (1 mg/kg IV) was acutely administered to these normal dogs, this dose of valsartan significantly inhibited the LV systolic pressure increase (148.0 ± 5.7% without valsartan, 99.0 ± 4.2% with valsartan, P<0.01). Chronic angiotensin II antagonism was also verified by the acute administration of angiotensin II (1 mg/kg IV) in 4w-paced valsartan-treated or untreated dogs. By comparison with that in the untreated dogs (n=4, 72.8 ± 4.5%), the angiotensin II–induced increase in LV pressure was significantly reduced in the valsartan-treated dogs (n=4, 33.0 ± 3.4%; P<0.01 versus untreated dogs).

Effects of Valsartan Treatment on Cardiac Performance, β-Receptor Density, and (Plasma and Tissue) Norepinephrine During the Development of Heart Failure

In valsartan-treated dogs with chronic RV pacing (4w-pacing), the contractile and relaxation functions at baseline were not significantly improved compared with the untreated dogs (Table). However, the positive inotropic response to dobutamine was larger in the valsartan-treated group than in
the untreated group (Figure 1A), indicating that contractility reserve was preserved in the valsartan-treated dogs.

The B\text{max} value for [3H]CGP12177 binding (fmol/mg) was lower in the untreated hearts than in the normal hearts, whereas it was restored to normal in the valsartan-treated hearts (normal, n=7, 57.7±2.0; untreated, n=7, 28.4±2.0; P<0.01 versus normal; valsartan-treated, n=7, 51.2±3.2; P<0.01 versus untreated). There was no significant difference in the K\text{d} for [3H]CGP12177 binding (nmol/L) among the 3 groups (normal, n=7, 0.41±0.09; untreated, n=7, 0.49±0.15; valsartan-treated, n=7, 0.46±0.13). Representative plots for [3H]CGP12177 binding are shown in Figure 1B.

Both plasma and cardiac tissue norepinephrine as well as the presynaptic uptake of norepinephrine by LV tissue were measured. As shown in Figure 1C, plasma norepinephrine, which was increased in the untreated paced dogs, was decreased to normal levels in the valsartan-treated paced dogs. Moreover, both tissue norepinephrine (which means the pooled norepinephrine in the nerve endings\textsuperscript{13}) and the presynaptic uptake of norepinephrine were well preserved in the valsartan-treated paced dogs.

To study the influence on cardiac function exerted by the norepinephrine released from nerve endings via presynaptic α\textsubscript{2}-receptors, we evaluated the effect of the α\textsubscript{2}-blocker yohimbine on various hemodynamic parameters in normal conscious dogs. Figure 2A shows the acute concentration-related effect of yohimbine on hemodynamic parameters in the presence or absence of valsartan (1 or 4 mg/kg per d for 3 days) in normal dogs. In valsartan-treated unpaced dogs, in which the peak dP/dt of LV pressure was decreased at baseline, it was increased by yohimbine to a level close to that seen without valsartan. In addition, as shown in Figure 2B, yohimbine increased the peak dP/dt only in 4w-paced valsartan-treated dogs, suggesting that when chronically administered, valsartan continues to inhibit the presynaptic release of norepinephrine.

Effects of Valsartan Treatment on Ca\textsuperscript{2+}-Handling Proteins in SR

Addition of 1 μmol/L thapsigargin to normal SR vesicles produced little Ca\textsuperscript{2+} leak, whereas addition of 30 μmol/L FK506 together with 1 μmol/L thapsigargin produced a pronounced leak (Figure 3A). In contrast, in failing (valsartan-untreated) SR vesicles, the addition of thapsigargin alone produced a prominent Ca\textsuperscript{2+} leak, but the addition of FK506 produced no additional increase. In SR vesicles from paced, valsartan-treated dogs, a substantial spontaneous Ca\textsuperscript{2+} leak was not observed, and FK506 had the same effect as in the normal SR (it greatly increased the Ca\textsuperscript{2+} leak).
In normal SR vesicles, addition of FK506, after Ca\(^{2+}\) uptake had reached a plateau, induced an increase in methylcoumarin-acetate (MCA) fluorescence that occurred at a faster rate than the Ca\(^{2+}\) leak seen in the same SR vesicles, but FK506 produced virtually no increase in MCA fluorescence in the failing (valsartan-untreated) SR vesicles (Figure 3B). In the valsartan-treated SR vesicles, FK506 induced an increase in the MCA fluorescence intensity, just as it did in the normal SR. We proposed previously\(^3\) that MCA fluorescence changes reflect the time course of conformational changes in RyR produced by the FK506-induced dissociation of FKBP12.6 from RyR. Therefore, the reappearance of an FK506-induced MCA fluorescence change in the valsartan-treated failing SR suggests that the conformational state of RyR was restored in these vesicles.

In valsartan-untreated failing SR vesicles, RyR was PKA-hyperphosphorylated, but this was reversed by valsartan treatment, the channel phosphorylation returning to the levels seen in the normal hearts (Figures 4A and 4B). The amount of RyR-associated FKB12.6 was in fact decreased by chronic RV pacing, but this decrease was largely prevented by valsartan treatment (Figure 4C). Moreover, the B\(_{max}\) value obtained for \(^{[3]}\)H dihydro-FK506 binding was significantly larger in the valsartan-treated vesicles than in the valsartan-untreated vesicles, although the former value was still less than that obtained for normal vesicles (Figure 4D). The ratio of the B\(_{max}\) for \(^{[3]}\)H dihydro-FK506 binding to that of the high-affinity \(^{[3]}\)Hryanodine binding was larger for the valsartan-treated failing vesicles (2.17±0.23, \(P<0.05\)) than for their valsartan-untreated counterparts (1.11±0.16), and the former value approached the one obtained for normal SR vesicles (3.75±0.21).

After 4 weeks of rapid RV pacing, both the SR Ca\(^{2+}\) uptake and the amount of SR Ca\(^{2+}\)-ATPase were decreased, and the decreases were smaller in the valsartan-treated group than in the untreated group (Figures 5A and 5B). Figure 5C compares the levels of Ser16-phosphorylated phospholamban (p-PLB) and total PLB (t-PLB) among the SR vesicles. There was no difference in the level of total PLB among the 3 groups, but there was a significant decrease in the basal level of phosphorylated PLB in the untreated failing SR vesicles. In the valsartan-treated SR vesicles, this level of phosphorylated PLB was restored back toward normal. A similar result was obtained for the ratio of p-PLB to t-PLB (Figure 5C).

**Contractile and Relaxation Functions During the Recovery Phase After Stopping Both Rapid RV Pacing and Valsartan Administration**

To eliminate both the acute detrimental effect of RV pacing and the acute effect of valsartan administration, we evaluated the contractile and relaxation functions during the recovery phase after stopping both RV pacing and valsartan administration. As shown in Figures 6A and 6B, LV contractile and relaxation functions improved more rapidly and more strongly in the valsartan-treated group than in the untreated group (improvement being a decrease in LV end-diastolic pressure, an increase in peak \(+\)dP/dt, a shortening of Tau, and a decrease in LV chamber size with improved wall motion). Moreover, as shown in Figure 6C, the tissue cyclic AMP level was significantly decreased in both valsartan-treated and -untreated groups compared with that in the normal group. However, in the recovery phase it was restored to normal in the valsartan-treated group.
Discussion

In contrast to the generally accepted favorable effects of ACE inhibitors in a variety of cardiac hypertrophy/failure models, there are some inconsistencies in the reported effects of angiotensin II–receptor blockade on LV function or remodeling.\textsuperscript{16} In the present study, we found that although valsartan failed to improve resting LV contractile function or to attenuate LV remodeling, SR function was corrected by this during the development of pacing-induced heart failure.

Because the pacing-induced downregulation of $\beta$-adrenergic receptors was inhibited by valsartan treatment, this effect of valsartan may be attributable in part to an inhibition of the presynaptic effect of angiotensin II on norepinephrine release, because angiotensin II is known to enhance the sympathetic release of norepinephrine.\textsuperscript{17} This idea is supported by the present findings that although both cardiac tissue norepinephrine and myocardial norepinephrine uptake were decreased in the failing hearts, they were restored by valsartan-treatment, and that yohimbine, known to be an $\alpha_2$-blocker promoting the release of norepinephrine from nerve endings,\textsuperscript{18} reversed the negative inotropic effect of valsartan in normal dogs (Figure 2A) and increased contractility only in valsartan-treated 4w-paced dogs (Figure 2B). These findings suggest that, in failing hearts, norepinephrine release from sympathetic nerve endings is increased and that this hyperadrenergic signal is transmitted into the cell, resulting in a hyperphosphorylation of RyR and an abnormal Ca\textsuperscript{2+} leak, and that valsartan, by acting on the
presynaptic angiotensin II receptor, inhibits norepinephrine release and stimulates norepinephrine uptake back into the synaptic pool, and thus less adrenergic signal is transmitted into the cell, reducing RyR-phosphorylation.

In heart failure, contractile and relaxation dysfunctions that develop within myocytes during the process of LV remodeling are likely to involve other factors besides alterations in the excitation-contraction coupling process, such as desensitization of β-adrenergic signaling and apoptosis and changes in extracellular matrix. Considering that valsartan failed to improve resting cardiac function and LV remodeling despite the improvement it induced in SR function, abnormalities in SR function may not necessarily be a cause of resting cardiac dysfunction but possibly a secondary response to impaired cardiac function during the development of pacing-induced heart failure.

The acute negative inotropic effect of valsartan (as seen in Figure 2A) may also be partly involved in the pathogenic process of the impaired resting cardiac function in valsartan-treated hearts, based on the following findings. When we examined the dogs’ cardiac performance after stopping both RV pacing and valsartan administration, the greater and faster recovery of hemodynamics was shown in the valsartan-treated group than in the valsartan-untreated group (Figure 6). Moreover, in this recovery phase, the tissue cyclic AMP was significantly restored toward the normal level in the

Figure 4. A and B, PKA-mediated phosphorylation of RyR confirmed by backphosphorylation. Nonspecific phosphorylation (not inhibited by PKA inhibitor) was subtracted, and the resulting value was divided by the amount of RyR2 protein (determined by immunoblotting and densitometry) and expressed as the inverse of the specific PKA-dependent [γ-32P]ATP signal (±SD). IP indicates immunoprecipitation; PKI, PKA inhibitor. C, Amount of RyR-bound FKBP12.6. D, Representative [3H]dihydro-FK506 bindings to SR vesicles. Inset, Scatchard replot of the binding data. *, normal group; •, valsartan-untreated 4w-paced group; ■, valsartan-treated 4w-paced group.
valsartan-treated group, whereas it remained significantly below normal in the untreated one.

Although valsartan did not improve resting cardiac function, valsartan treatment may increase the contractility reserve, as suggested by dobutamine responses (Figure 1A). This improved hemodynamic response may be caused by normalization of Ca²⁺ regulatory process, and this would lead to improved exercise tolerance. Also, inhibition of an aberrant SR Ca²⁺ leak, which can trigger arrhythmias by initiating delayed afterdepolarizations, may lead to a better prognosis in patients with heart failure.

In a recent multicenter trial (Val-HeFT), valsartan decreased the other primary end point (combined morbidity/mortality) by 13.2% in patients with heart failure. However, post hoc observation revealed an adverse effect on mortality and morbidity in the subgroup receiving valsartan together with both an ACE inhibitor and a β-blocker. Taken together with the present data, this suggests that the β-adrenergic blocking effect may be exaggerated by combining valsartan with a β-blocker, leading to a serious deterioration of cardiac performance in some patients with heart failure.

Before we can firmly draw conclusions, several questions remain to be answered. First, there are some results that seem to challenge the concept that hyperphosphorylation-induced dissociation of FKBP12.6 is an important factor in abnormal Ca²⁺ homeostasis in heart failure. Jiang et al²¹ have reported that no difference was detected in the degree of RyR2 phosphorylation in pacing-induced canine heart failure. In

**Figure 5.** A, Amount of SR Ca²⁺ ATPase in normal, untreated failing, and valsartan-treated failing SR vesicles. B, ATP-dependent Ca²⁺ uptake when [Ca²⁺] content was 0.1, 0.3, 1.0, or 10 μmol/L. ○, normal group; ●, untreated 4w-paced group; ■, valsartan-treated 4w-paced group. Data are mean±SD. **P<0.01 vs normal SR. #P<0.05, ##P<0.01 vs 4w-paced untreated group. C, Representative Western blot analysis of Ser16-phosphorylated PLB (p-PLB) and total PLB (t-PLB). D, Densitometric analysis of the Western blot. The p-PLB values (sum of pentamer and monomer, in arbitrary units) were normalized with respect to the t-PLB values (sum of pentamer and monomer, in arbitrary units). Data are mean±SD.
their study, there was no significant difference in the $B_{\text{max}}$ of $[^{3}H]$ryanodine binding between normal and failing hearts, although we observed the consistent decrease in the $B_{\text{max}}$ by approximately 50% in failing hearts. Therefore, the degree of heart failure may be severer in our models than in theirs. Also, these discrepancies may result from different experimental techniques. Nevertheless, the importance of hyperphosphorylation of RyR2 in influencing myocyte contractility in heart failure needs and is certain to receive additional investigation. Second, we should address why RyR was PKA-hyperphosphorylated whereas PLB was PKA-hypophosphorylated in failing hearts. Loss of RyR-associated phosphatases (despite the increase in cytosolic phosphatases) might explain the difference in PKA phosphorylation level between RyR and PLB. Third, we should state that the tachycardia-induced model of heart failure may not be well suited for studies of the cause of progression of heart failure, because therapies have no impact on the fundamental basis of the problem, i.e., incessant tachycardia.
In conclusion, during the development of pacing-induced heart failure, valsartan maintained the density of β-adrenergic receptors and stabilized the channel gating of RyR, with an improvement in the SR Ca\(^{2+}\) uptake function. However, it failed to improve resting cardiac function in this model. These findings suggest that there is a separation between changes in β-adrenergic receptor–mediated signaling (and concurrent effects on SR function) and changes in mechanical performance in some types of failing hearts.

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