Sarcoplasmic reticulum function in determining atrioventricular contractile differences in rat heart

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Minajeva, Ave, Allen Kaasik, Kalju Paju, Enn Seppet, Anne-Marie Lompré, Vladimir Veksler, and Renée Ventura-Clapier. Sarcoplasmic reticulum function in determining atrioventricular contractile differences in rat heart. J. Physiol. 273 (Heart Circ. Physiol. 42): H2498-H2507, 1997.—The relationships between the contractile characteristics and the sarcoplasmic reticulum (SR) function of rat atrial and ventricular trabeculae were compared. The isometric developed tension (DT) and the rates of contraction (+dT/dt) and relaxation (−dT/dt) normalized to cross-sectional area were 3.7, 2.2, and 1.8 times lower, respectively, in intact atrial strips compared with ventricular strips, whereas +dT/dt and −dT/dt (normalized to DT) were 2.3 and 2.8 times higher, respectively, in atria. Atria exhibited a maximal potentiation of DT after shorter rest periods than ventricles and a lower reversal for prolonged rest periods. Caffeine-induced tension development in intact atrial myocardium compared with ventricles and a lower amount of mRNA encoding the myosin heavy chain, calsequestrin, and the ryanodine receptor was similar in both tissues. Thus a lower amount of the fast α-myosin heavy chain (MHC) isoform com-
pared with ventricles. However, this seems not to be an explanation in small mammals such as rats, in which both atria and ventricles express α-MHC in a similarly high proportion (28), but atria still contract faster than ventricles (16). While addressing this issue, we recently observed that skinned left atrial and ventricular preparations from adult rat heart developed similar levels of maximal force and stiffness, displaying also similar Ca²⁺ sensitivity and tension kinetics (31). Conversely, faster cross-bridge kinetics have been observed in right atria of hyperthyroid rats compared with ventricles and have been attributed to the differences in myosin light chain content between the two tissues (11). Several observations, however, suggest that differences in Ca²⁺ handling at the level of the sarcoplasmic reticulum (SR) may contribute to the atrioventricular differences in contractile function. In mouse heart, both volume fraction and surface area of total SR per cell volume are higher in atria, mainly due to a higher longitudinal SR content (10). Therefore, atrial contraction has been proposed to be more dependent on Ca²⁺ release by the SR than ventricular contraction (1, 9, 22). Atrial SR has been shown to exhibit a 4.2-fold lower ratio of phospholamban to Ca²⁺-adenosinetriphosphatase (ATPase) than ventricular SR, and the lower phospholamban-to-Ca²⁺-ATPase ratio has been sug-
gested to increase SR Ca²⁺ uptake and, consequently, lead to faster relaxation in rat atria (17). Unlike in ventricular cardiomyocytes, a nonsynchronous and bi-
phasic Ca²⁺-induced Ca²⁺ release has been observed in atrial cardiomyocytes (8, 14).

Atrioventricular differences in contractile function may also reflect the different metabolic profiles of these tissues. In comparison with ventricles, atria are charac-
terized by lower activities of glycolytic and citric acid cycle enzymes (4). Lower oxidative capacities in atria are associated with decreased activities of both total creatine kinase (CK) and mitochondrial CK (mito-CK) compared with ventricles (31). It has also been shown in atria that mito-CK is not coupled to the adenine nucleotide translocase (31). These findings suggest fundamentally different mechanisms of energy trans-
port in atrial compared with ventricular cells. In this respect, a question arises concerning the interactions between CK and the SR Ca²⁺-ATPase in atria. The MM form of CK (MM-CK) bound to the myofilaments exhibited the same efficacy in controlling the ATP-to-ADP ratio in atria and ventricles (31). Characterization of SR function in isolated membrane preparations and in
skinned fibers from ventricular myocardium has revealed a functional coupling between SR Ca\textsuperscript{2+} uptake and MM-CK bound to the SR membranes (18, 24). However, nothing is known about the role of CK in SR and MM-CK bound to the SR membranes (18, 24).

This work was undertaken to study and compare the relationships between the contractile parameters, the expression of SR proteins, the SR function, and the functional characteristics of MM-CK bound to SR in atria and ventricles from rats. It was found that, compared with ventricles, atria exhibit a higher expression of SR Ca\textsuperscript{2+}-ATPase (SERCA) 2a associated with faster SR Ca\textsuperscript{2+} uptake but prolonged caffeine-induced Ca\textsuperscript{2+} release. These properties of SR are accompanied by a faster rate of filling during interbeat pauses as well as a reduced loss of SR Ca\textsuperscript{2+} during pause decay in intact atria. At the same time, the SR calsequestrin (Cals) and ryanodine receptor (RyR) expression as well as the SR capacity of releasable Ca\textsuperscript{2+} appear similar in atria and ventricles. Like in ventricles, the SR Ca\textsuperscript{2+} uptake is highly dependent on ATP generated locally in the CK reaction, thus indicating a coupling of MM-CK to the SR Ca\textsuperscript{2+} pump in atria.

MATERIALS AND METHODS

Muscle preparations. Wistar rats of both sexes (average body wt 260 g) were treated according to the recommendations of the institutional animal care committee (INSERM, Paris, France). They were anesthetized by intraperitoneal injection of urethane (0.2 g/100 g body wt). For contractility measurements, papillary muscles from right ventricles or fibers from papillary muscles of left ventricles and atria with similar cross-sectional areas were dissected in a solution containing (in mM) 120 NaCl, 5.4 KCl, 0.6 CaCl\textsubscript{2}, 0.42 NaH\textsubscript{2}PO\textsubscript{4}, 1.05 MgCl\textsubscript{2}, 5 glucose, 30 2,3-butanedione monoxime, 0.05 Na\textsubscript{2} EDTA, and 20 tris(hydroxymethyl)aminomethane (Tris)-HCl, gassed with 100% oxygen, pH 7.4.

Fibers to be used for permeabilization of the sarcolemma (diameter 150–240 µm) were dissected from left ventricular papillary muscle or from left atria in ice-cold zero-Ca\textsuperscript{2+} Krebs solution containing (in mM) 118 NaCl, 4.7 KCl, 25 NaH\textsubscript{2}CO\textsubscript{3}, 1.2 KH\textsubscript{2}PO\textsubscript{4}, and 1.2 MgSO\textsubscript{4} equilibrated with 95% O\textsubscript{2}-5% CO\textsubscript{2}. Specific permeabilization of the sarcolemma was obtained by incubating the fibers in relaxing solution A (see Table 1) containing 50 µg/ml saponin in the presence of 20 µM leupeptin at +4°C for 30 min (13).

Contractile function measurements. Muscle preparations with silk thread tied to one end were placed horizontally in a solution containing (in mM) 120 NaCl, 5.9 KCl, 2.5 CaCl\textsubscript{2}, 1.2 MgCl\textsubscript{2}, 11 glucose, 1.1 mannitol, and 5 N-2-hydroxyethylpiperazine-N\texttextsubscript{2}-(2-hydroxyethyl)ethanesulfonic acid, pH 7.4 at 30°C, aerated with 100% O\textsubscript{2}. The isometric contractile parameters of muscle preparation were registered at the peak of the length-tension curve using on-line PC-AT 486 with Atrium software designed by Dr. U. Braun. The length of each fiber was measured by means of a micrometer in a dissecting microscope. At the end of the experiment, the fiber was cut off between the silk thread and the needle and weighed. Mean cross-sectional area for each preparation was calculated from the weight and length of the trabeculae, assuming a muscle density of one. Tension values were expressed in millinewtons per square millimeter.

The dependence of postrest potentiation on the rest interval at extracellular Ca\textsuperscript{2+} concentrations ([Ca\textsuperscript{2+}]\textsubscript{o}) of 1 and 2.5 mM was used to compare the SR function in intact myocardium. In these experiments, the basic stimulation at 1 Hz was interrupted for 3–600 s. In each preparation, the values of the developed tension (DT) of the first postrest switches were normalized to the maximal value of potentiation, usually gained after a 60- to 120-s pause.

Preparation of tissue homogenates and estimation of SR Ca\textsuperscript{45} uptake. Rats were anesthetized with thiopental sodium (50 mg/kg), and hearts were excised and rinsed rapidly in ice-cold isotonic saline solution. The whole atrial aurides and ventricular apex region were isolated and weighed. The homogenization was carried out with an Ultra-Turrax homogenizer (3 × 20 s, 24,000 revolutions/min) in 50 vol of ice-cold homogenization buffer containing (in mM) 250 sucrose, 20 Tris (pH 6.8), 2 MgCl\textsubscript{2}, 0.01 leupeptin, 0.01 phenylmethylsulfonyl fluoride, 1 dithiothreitol, and 2 benzamidine. The final homogenate was further treated with a glass-glass homogenizer (10 strokes).

The Ca\textsuperscript{2+} accumulation in the tissue homogenate was determined at 30°C in stirred medium containing (in mM) 6 ATP, 6 MgCl\textsubscript{2}, 120 KCl, 3 sodium azide (NaN\textsubscript{3}), 60 midazole (pH 7.0), 6 potassium oxalate, and a 45Ca-labeled CaCl\textsubscript{2}-ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N’-tetraacetic acid (EGTA) buffer containing 0.58 mM EGTA. Appropriate concentrations of CaCl\textsubscript{2} were added to the medium to obtain different free Ca\textsuperscript{2+} concentrations ([Ca\textsuperscript{2+}]\textsubscript{o}). The reaction was started by addition of 50 µl of homogenate per 0.5 ml of medium. After a 2-min incubation, the samples were filtered through 0.45-µm Schleicher & Schuell (Keene, NH) glass microfiber filters using a vacuum pump. Radioactivity associated with the membranes was counted in Optiphase “HiSafe” 3 (Wallac, Turku, Finland). After subtraction of unspecified binding, the Ca\textsuperscript{2+} uptake by the homogenate was entirely blocked by 50 µM cyclopiazonic acid, a specific inhibitor of SR Ca\textsuperscript{2+}-ATPase that shows that Ca\textsuperscript{2+} uptake was restricted to the SR Ca\textsuperscript{2+} pump. Protein concentration in the homogenate was determined by the biuret method and was between 104 and 166 µg per uptake assay.

Estimation of SR Ca\textsuperscript{2+} uptake in saponin-permeabilized fibers. SR Ca\textsuperscript{2+} uptake in permeabilized fibers was estimated by analyzing the tension transients due to caffeine-induced Ca\textsuperscript{2+} release after various periods of SR loading (13, 30), as described recently (24). The fibers were mounted between a length-adjustment device and a force transducer (AE 801, Aker’s Microelectronics, Horten, Norway). Fibers were immersed in 2.5-ml chambers arranged around a disk. The chambers were placed in a temperature-controlled bath positioned on a magnetic stirrer. All experiments were performed at 22°C. The isometric force measurements were calculated as described previously and are listed in Table 1. All solutions contained (in mM) 0.8 free Mg\textsuperscript{2+}, 30.6 Na\textsuperscript{+}, 60 N-N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 7.1), 3.16 MgATP, 12 phosphocreatine (PCr), and 0.3 dithiothreitol. The Pionic strength was adjusted to 160 mM with potassium methanesulfonate. All solutions contained 2 mM NaN\textsubscript{3} to inhibit possible Ca\textsuperscript{2+} uptake by mitochondria and 20 µM leupeptin to inhibit the proteases. To study the energy requirement for SR Ca\textsuperscript{2+} loading, solutions with modified adenine nucleotides and PCR content were designed.
Tmax. The data from each fiber were fitted by the Hill equation (at pCa 4.5). DT at each pCa was normalized with respect to coefficient (uptake in different fibers or loading conditions).

| Table 1. Composition of solutions for estimating Ca\(^{2+}\) uptake in skinned cardiac fibers |
|-----------------------------|-----|-------|------|
| Solutions                  | pCa | EGTA, mM | Caffeine, mM |
| Relaxing A                 | 9.0 | 10     |      |
| Relaxing A\(_1\)           | 9.0 | 10     |  5   |
| Activating B               | 4.5 | 10     |      |
| Loading C                  | 6.5 | 10     |      |
| Prerelease D               | 9.0 | 0.25   |      |
| Release R\(_1\)            | 9.0 | 0.2    |  5   |

All solutions contained (in mM) 0.8 free Mg\(^{2+}\), 30.6 Na\(^{+}\), 60 N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 7.1), 3.16 MgATP, 12 phosphocreatine, 0.3 dithiothreitol, 2 sodium azide, and 20 µM leupetin. Theionic strength was adjusted to 160 mM with potassium methanesulfonate.

At the beginning of each experiment, the fiber was stretched to 120% of the slack length in the relaxing solution A. The maximal Ca\(^{2+}\)-activated tension (T\(_{max}\)) was estimated in the activating solution B, pCa 4.5. The fiber was then relaxed in solution A. To empty the SR of Ca\(^{2+}\), 5 mM caffeine was applied for 2 min in the relaxing solution (A\(_1\)) followed by the washout of caffeine for 2 min in the same solution (A). SR loading was carried out at pCa 6.5 and 10 mM [EGTA] (solution C) for different time periods, so that under control conditions the peak force never reached the upper saturating part of the pCa-tension relationship and the Ca\(^{2+}\) release was in the quasi-linear part of the pCa-tension relationship. To wash out Ca\(^{2+}\) and EGTA after loading, the prerelease solution (D) containing 0.25 mM [EGTA] was applied for 1.5 min. The fiber was then passed into another prerelease solution (E) with 0.2 mM [EGTA] for 30 s. Finally, Ca\(^{2+}\) was released from the SR by applying 5 mM caffeine in a solution of the same composition (R\(_1\)), which resulted in a tension transient. The peak of relative tension (T/T\(_{max}\)) and the area released from the SR by applying 5 mM caffeine in a solution identical to those of the release (except that 10 mM EGTA was present to adequately buffer free Ca\(^{2+}\)) was obtained at the end of each experiment by sequentially exposing the fibers to a set of solutions with decreasing pCa until T\(_{max}\) was reached (at pCa 4.5). DT at each pCa was normalized with respect to T\(_{max}\). The data from each fiber were fitted by the Hill equation using linear regression analysis, and the Ca\(^{2+}\) required to produce 50% of maximal activation (pCa\(_{50}\)) and the Hill coefficient (n\(_h\)) were determined. The same Ca\(^{2+}\)-release protocol was always applied to enable comparison of SR Ca\(^{2+}\) uptake in different fibers or loading conditions.

Evaluation of the role of bound CK in SR Ca\(^{2+}\) uptake was done by analyzing tension transients due to caffeine-induced Ca\(^{2+}\) release, as described above.

Isolation of total RNA and mRNA dot-blot analysis. Eight hearts were collected from adult Wistar rats. The atria were isolated, and the ventricles were cut into pieces. The tissues were blotted dry, frozen in liquid nitrogen, and kept at –80°C until RNA preparation. Both atria from each rat and a piece of ventricle with approximately the same weight were used for RNA preparation. Total RNA was extracted by the guanidine isothiocyanate procedure using RNA quick (Bioprobe) and kept at –20°C in 70% ethanol, 0.3% sodium acetate, pH 5.2. Specific mRNA species were quantified by slot-blot hybridization. One, two, and four micrograms of total RNA from atria and ventricles as well as from liver and fast skeletal muscle (extensor digitorum longus) and yeast tRNA were denatured in 15× standard saline citrate (SSC; 1× SSC contained 0.15 M sodium chloride and 0.015 M sodium citrate) and 3% formaldehyde at 65°C for 15 min and rapidly blotted onto a nylon membrane. Liver, skeletal muscle RNA, and tRNA were used as negative controls to check for specificity of the various probes. The samples were directly spotted onto the nylon membrane using a minifold apparatus (Schleicher & Schuell). The RNA was cross-linked to the membrane by ultraviolet irradiation, and the membranes were prehybridized at 42°C for >4 h in the presence of 50% formamide, 0.1% bovine serum albumin, 0.1% Ficol, 0.1% polyvinylpyrrolidone, 0.05 M sodium phosphate (pH 6.5), 5× SSC, 0.1% sodium dodecyl sulfate (SDS), and 250 µg/ml salmon sperm DNA.

The specific SERCA2 mRNA was detected using a probe [from nucleotide (nt) 2616 to 3120] of the rat heart Ca\(^{2+}\)-ATPase mRNA (20). The rat RyR probe (RyR-2) was obtained by reverse transcription of rat cardiac total RNA and subsequent amplification of the RyR-2 mRNA using primers derived from the sequence of the rabbit cardiac RyR-2 (25). The probe extends from nt 8604 to 9144 of the rabbit sequence (25) with few silent mutations. The dog Cals probe was a gift from Dr. B. Nadal-Ginard and was used as described by Lompré et al. (21). The α-MHC probe has been previously described by Schiaffino et al. (29). The cDNA probes were labeled by use of random primers, DNA polymerase I (Klenow fragment) and [α-32P]dATP (3,000 Ci/mmol), and the specific activity was 1×3×10^6 disintegrations/min (dpm)/µg. Hybridization was done in the same conditions as prehybridization. Excess of probe was eliminated by washing in 0.5× SSC at 55°C for SERCA2, at 60°C for RyR-2, and at 42°C for Cals and in 1× SSC at 45°C for MHC.

After each hybridization, the blots were dehybridized by boiling in 0.1% SDS and then rehybridized as described above. To normalize to the amount of total RNA present on the membrane, the blots were rehybridized with a 24-mer oligonucleotide complementary to the rat 18S ribosomal RNA. The oligonucleotide was labeled at its 5’ end by use of T4 polynucleotide kinase and [α-32P]dATP and diluted with cold oligonucleotide to a specific activity of 10^6 dpm/μg. It was hybridized in the medium described above but in the absence of formamide. The washing conditions were 2× SSC at room temperature. After washing, the membranes were exposed to X-ray film for 1 day to 1 wk. Unsaturated autoradiograms were analyzed by densitometry (Molecular Dynamics). The specific mRNA level was corrected for the total RNA present on the membrane by calculating the ratio of the signals obtained with the specific probe and the 18S probe for the
three dilutions of each sample. Specific mRNA levels were expressed in arbitrary units (AU) as means ± SE. The relative proportion of SERCA2 and SERCA2b mRNA was determined by ribonuclease (RNase) protection assay using the SERCA2 probe described above and the Ambion kit protocol (Clinisciences, Montrouge, France).

Chemicals. Caffeine was purchased from Merck-Clevenot. PCr (Neoton, Schiapparelli Searle, Turin, Italy) was a kind gift from Prof. E. Strumia. Other chemicals were obtained from Sigma Chemical.

Statistical Analysis. The data are expressed as means ± SE or as representative tracings in a single experiment. Statistical analysis is presented in RESULTS. Differences at P < 0.05 were considered significant.

RESULTS

Contractile parameters. Figure 1 shows that atrial contractions are characterized by a weaker twitch DT but a faster time course than ventricular contractions. The mean values of contractile characteristics are presented in Table 2 and compared using unpaired Student’s t-test. DT in atrial preparations was 3.7 times less than that in ventricular preparations. The time to peak tension (TPT) and the half-relaxation time (RT50) were significantly less in atria. The maximal rates of contraction (+dT/dt) and relaxation (−dT/dt) also appeared to be lower in atria than in ventricles. However, if normalized to DT, these parameters [(+dT/dt)/DT and (−dT/dt)/DT] became higher in atria, evidencing faster contractile kinetics.

Postrest potentiation. Postrest potentiation of twitch tension was compared in atria and papillary muscles at two external [Ca2+] (1 and 2.5 mM) to assess the function of SR in providing activator Ca2+ in vivo (3, 19; for review see Ref. 9). The absolute mean values of pretest steady-state and maximal potentiation in both tissues are presented in Table 3. In Fig. 2, values were normalized to maximal potentiation to compare the two tissues. The values were analyzed using analysis of variance (ANOVA) followed by Dunnett’s test. Figure 2A shows that, at 1 mM [Ca2+]o, increasing the rest duration was accompanied by potentiation of the first postrest twitch in both atrial and papillary muscle preparations. However, the maximal level of potentiation was achieved after significantly shorter rest intervals (15 s) in atrial than in papillary muscles (60 s). In addition, the magnitude of potentiation was less in atria than in ventricles. Further increase in rest duration led to significant reversal of potentiation in papillary but not in atrial muscles. Figure 2B demonstrates that these differences between atrial and papillary muscles were abolished when [Ca2+]o was increased from 1 to 2.5 mM.

Oxalate-supported 45Ca2+ uptake in tissue homogenates. The oxalate-supported 45Ca2+ uptake rates were estimated in tissue homogenates in conditions (see MATERIALS AND METHODS) under which the [Ca2+]o has been previously defined to be restricted to SR vesicles (26). In our experiments, the specific SR Ca2+-ATPase inhibitor cyclopiazonic acid (30 µM) inhibited ~98% of Ca2+ uptake. This also confirms that the intrinsic SR Ca2+ uptake was assayed.

The net SR Ca2+ uptake rates in tissue homogenates of both atria and ventricles were assessed in the presence of oxalate over a wide range of free [Ca2+]o, corresponding to the range of cytosolic free [Ca2+] during the contraction-relaxation cycle (Fig. 3). Data were fitted using the Hill equation, and the results were compared using Student’s t-test. The net maximal Ca2+ uptake rate was more than twofold higher in atria than in ventricles (4.17 ± 0.72 and 2.03 ± 0.42 nmol Ca2+ mg tissue protein−1 min−1, respectively; P < 0.05). However, the free Ca2+ concentration that produces

![Image](http://ajpheart.physiology.org/Downloadedfromhttp://ajpheart.physiology.org/)

### Table 2. Mean characteristics and contractile parameters obtained at 1-Hz stimulation, [Ca2+]o 2.5 mM, of fibers dissected from rat atria and ventricles

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>S, mm²</th>
<th>DT, mN mm²</th>
<th>Resting Tension, mN mm²</th>
<th>TPT, ms</th>
<th>RT50, ms</th>
<th>+dT/dt, (mN/s mm²)</th>
<th>−dT/dt, (mN/s mm²)</th>
<th>(+dT/dt)/DT, s⁻¹</th>
<th>(−dT/dt)/DT, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atria</td>
<td>13</td>
<td>0.19 ± 0.02</td>
<td>7.4 ± 0.8</td>
<td>2.1 ± 0.3</td>
<td>71 ± 1</td>
<td>32 ± 2</td>
<td>173 ± 24</td>
<td>120 ± 13</td>
<td>26.2 ± 4.6</td>
<td>18.7 ± 2.9</td>
</tr>
<tr>
<td>Ventricles</td>
<td>11</td>
<td>0.19 ± 0.02</td>
<td>26.1 ± 1.6</td>
<td>5.7 ± 0.8</td>
<td>145 ± 3</td>
<td>104 ± 5</td>
<td>295 ± 16</td>
<td>173 ± 13</td>
<td>11.4 ± 0.3</td>
<td>6.7 ± 0.5</td>
</tr>
</tbody>
</table>

P < 0.0001

Values are means ± SE; n, no. of fibers; [Ca2+]o, extracellular Ca2+ concentration; S, cross-sectional area; DT, developed tension; TPT, time to peak tension (from the moment of stimulation to peak twitch tension development); RT50, half-relaxation time; +dT/dt, maximal rate of tension development; −dT/dt, maximal rate of relaxation; NS, not significant.
half-maximal activation of the SR Ca\(^{2+}\) pump (0.22 ± 0.03 and 0.22 ± 0.04 µM in atria and ventricles, respectively) as well as the \(n_H\) (1.55 ± 0.13 and 1.68 ± 0.34 in atria and ventricles, respectively) were similar in atria and ventricles.

Ca\(^{2+}\) uptake in skinned cardiac fibers. To compare the SR Ca\(^{2+}\) handling in atrial and ventricular myocardium in situ, saponin-permeabilized fibers with similar mean diameters were used. Table 4 shows that atrial and ventricular preparations exhibited similar values of \(T_{\text{max}}\) normalized per cross-sectional area and of myofilament Ca\(^{2+}\) sensitivity in the presence of 5 mM caffeine. In addition, no effect of caffeine on \(T_{\text{max}}\) was observed (results not shown).

The similar responsiveness of myofibrils from atrial and ventricular preparations to Ca\(^{2+}\) in the presence of 5 mM caffeine allows us to compare the time courses of caffeine-induced tension transients in the two tissues. The SR Ca\(^{2+}\) uptake was estimated by loading tissues with Ca\(^{2+}\) for different time periods and subsequent liberation by caffeine. Figure 4 shows that, after a 10-min load at pCa 6.5, a typical caffeine-induced contracture in atrial fibers was characterized by lower peak tension and a substantial tonic component of the contracture, whereas ventricular muscle promptly returned to baseline tension. In Fig. 5, mean data as a function of loading time are presented and compared using ANOVA followed by Dunnett's test. Figure 5A shows that, in response to increased periods of loading, the peak tension of caffeine-induced contractures increased in both atrial and ventricular fibers. However, the peak tension values normalized to \(T_{\text{max}}\) reached a lower level (50%) in atrial than in ventricular fibers (80%). The \(R_{50}\) of caffeine-induced contracture increased progressively with the loading time in both tissues (Fig. 5B), reaching, however, significantly higher levels in atrial than in ventricular preparations at loading times >10 min. To eliminate the possibility that these differences were due to limited diffusion of caffeine at a concentration of 5 mM, application of 25 mM caffeine was used in some experiments. The results (not shown) indicated that the higher caffeine did not diminish the atrioventricular differences in the time course of tension transient. In addition, when a second

Table 3. Absolute mean values of maximally potentiated and steady-state twitch amplitudes in atria and ventricles

<table>
<thead>
<tr>
<th>[Ca(^{2+})](_o)</th>
<th>Atria (n)</th>
<th>Ventricle (n)</th>
<th>Atria (n)</th>
<th>Ventricle (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>8</td>
<td>3.6 ± 0.6</td>
<td>8.27 ± 2.0</td>
<td>10</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>7</td>
<td>8.4 ± 0.9</td>
<td>12.7 ± 2.1</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in mN/mm\(^2\); n, no. of fibers. *P < 0.05; †P < 0.01; ‡P < 0.001, ventricles vs. atria.

Fig. 3. Sarcoplasmic reticulum (SR) Ca\(^{2+}\) uptake rate in 4 different preparations of atrial (●) and ventricular (○) homogenates at different pCa (expressed in nmol Ca\(^{2+}\)·min\(^{-1}\)·mg protein\(^{-1}\)). Each curve was drawn using mean values of 4 preparations. Continuous curves obtained from Hill equation were fitted by least-square fitting procedure.

Table 4. Characteristics of saponin-permeabilized atrial and ventricular fibers in presence of 5 mM caffeine

<table>
<thead>
<tr>
<th>n</th>
<th>Diameter, µm</th>
<th>(T_{\text{max}}), mN/mm(^2)</th>
<th>pCa(_{50})</th>
<th>(n_H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atria</td>
<td>15</td>
<td>210 ± 14</td>
<td>21.9 ± 5.1</td>
<td>5.85 ± 0.03</td>
</tr>
<tr>
<td>Ventricle</td>
<td>20</td>
<td>207 ± 7</td>
<td>19.1 ± 1.4</td>
<td>5.82 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of fibers. \(T_{\text{max}}\), maximal Ca\(^{2+}\)-activated tension; pCa\(_{50}\), pCa for half-maximal activation; \(n_H\), Hill coefficient. pCa\(_{50}\) and \(n_H\) have been calculated using nonlinear fit of the Hill equation for each fiber. NS, difference not significant.

Fig. 2. Rest potentiation in atria (●, n = 8) and ventricles (○, n = 10) at 1 (A) and 2.5 mM (B) external Ca\(^{2+}\) concentrations. Values have been normalized to maximal potentiation. **P < 0.01; ***P < 0.001, ventricles vs. atria.
application of 5 mM caffeine was made immediately after the first, no increase in tension was observed in either tissue, suggesting that the slow phase of tension transient in atria was due to a slow phase of Ca^{2+} release. Further analysis of tension-time integrals ($ST$) calculated over the whole tension transient revealed that the area under the caffeine-induced tension transient was the same in atrial and ventricular fibers (Fig. 5C). Thus, despite the different shape of the caffeine-elicited tension transients and a faster SR Ca^{2+}-uptake rate in atria (Fig. 3), atria seemed to exhibit at least equal SR Ca^{2+} capacity in situ as ventricles.

Energy dependence of SR Ca^{2+} uptake in situ in atria. To evaluate the efficiency of the MM-CK bound to SR in providing the SR Ca^{2+} pump with ATP in situ, we compared the SR Ca^{2+} loading in the presence of MgADP and PCr (when ATP generated by the bound CK was the only source of energy for the Ca^{2+} pump) with that supported by MgATP alone. The level of SR Ca^{2+} loading in the presence of 3.16 mM MgATP and 12 mM PCr served as control. Data were compared using ANOVA followed by Dunnett's test. Figure 6A shows that when SR was loaded either in the presence of ADP and PCr or ATP and PCr the time dependence of relative peak tension for these two conditions had similar shape, both reaching their equivalent maximal values (51 and 47% of $T_{max}$, respectively) within 1 min. Further increase in loading time did not alter the values of $T_T_{max}$. In contrast, the curve obtained in the presence of ATP alone tended to decrease if the time of loading was prolonged for >1 min. As a result, the values of $T_T_{max}$ became significantly less than those in control conditions.

Figure 6B shows the dependency of the tension-time integral on the time of loading for different conditions of energy supply. It can be seen that $S_T$ increased progressively with the time of loading, being 11.2 ± 3.2 mN·s/mm² in the presence of ATP and PCr and not significantly different when ATP was replaced by ADP for a 7-min load. In contrast, when SR was loaded in the presence of ATP alone, the value of $S_T$ dramatically decreased (1.31 ± 0.16 mN·s/mm², $P < 0.05$) for the same loading time. These results show that the amount of Ca^{2+} released from the SR by caffeine was much higher in conditions when SR Ca^{2+} uptake was supported by ATP produced by the SR-bound CK than when externally added ATP was used as the only source of energy for the SR Ca^{2+} pump.

Expression of mRNA. To relate the observed differences in SR function between the two cardiac tissues to the level of expression of the principal SR proteins (SERCA, RyR, and Cals) and the $\alpha$-MHC, the tissue amounts of corresponding mRNA relative to 18S were estimated by slot blot (Fig. 7, A and B). Figure 7A shows that no signal was observed with any of our probes with liver RNA, whereas 18S RNA was present, indicating no nonspecific binding of the probes. This is also attested by the negative signal in tRNA samples. As expected, the $\alpha$-MHC and the RyR-2 probes were specific for cardiac RNA and were not detected in skeletal muscle, whereas the SERCA2 and Cals mRNA were present in both muscle types. Quantification of slot blot (Fig. 7B) indicates that the expression of the $\alpha$-MHC relative to 18S was not significantly different between atria and ventricles [1,000 ± 90 and 974 ± 81 AU, respectively]. Relative to 18S RNA, the content of RyR and Cals mRNAs in the two tissues was not different, whereas the amount of SERCA2 mRNA was
38% higher in atria than in ventricles (220 ± 21 and 160 ± 18 AU, respectively, P < 0.05). The relative proportion of SERCA2a and SERCA2b mRNA was determined by RNase protection analysis. As already shown in the ventricle, the majority of the SERCA2 mRNA was of the 2a type (21). An identical pattern was observed in the atria (data not shown). Relative to α-MHC mRNA, the amount of SERCA2 mRNA was lower in ventricles than in atria (0.165 ± 0.010 and 0.234 ± 0.049 AU, respectively), but the difference did not reach significance. This suggests that higher SR Ca2+ pump activity was due to a higher expression of pump protein in atria than in ventricles.

**DISCUSSION**

Contractile parameters in atria and ventricles. In comparison with papillary muscles, atrial preparations exhibited smaller absolute values of DT. However, the kinetic parameters (TPT and RT50) and +dT/dt and −dT/dt normalized to DT showed that peak isometric tension in atria reached its maximum and relaxed faster than in ventricles, in accordance with earlier studies (1, 2, 17). A low DT in left atria is strikingly in contrast to the observation that both maximum Ca2+-activated tension and Ca2+ sensitivity of myofibrils were similar in atrial and ventricular saponin-treated preparations (Table 3) (31). This could be the result of a smaller amount of activator Ca2+ in intact atria compared with ventricles. On the other hand, the process could be limited also by a higher rate of Ca2+ resorption by SR.

Rest potentiation. Rest potentiation has been suggested to result from the increase in Ca2+ release from the SR as a result of either the increase in SR Ca2+ content in rat cardiomyocytes (3, 7, 10, 19) or the increase in fractional SR Ca2+ release (6, 12). Our data indicate that, at low [Ca2+]i, although relative potentiation is lower in atria, the mechanisms responsible for maximal postrest potentiation need about four times less time to become saturated in atrial (15 s) than in ventricular (60 s) myocardium. This suggests that SR Ca2+ filling saturates earlier and faster in atria. These features of atrial myocardium could be associated with a faster SR Ca2+-uptake rate, although it is not clear whether the amount of SR-releasable Ca2+ is indeed increased. An increase in rest duration for 10 min led to significant reversal of potentiation in papillary muscles but not in atria. The decline in rest potentiation and rest decay has been attributed to SR Ca2+ loss due to its extrusion through the sarcolemmal Na/Ca exchanger (6, 9). This may indicate that the SR in rat ventricular myocardium loses more Ca2+ during pauses. As for rest potentiation, this could be attributed to a faster SR Ca2+-uptake rate in atria. On the other hand, atrial myocytes are characterized by higher intracellular sodium concentration due to a lower Na+/K+ ATPase content (33). This may lead to less pronounced Ca2+ extrusion via the Na/Ca exchanger, resulting in the less pronounced rest decay. Indeed, under high [Ca2+]o, which minimizes the Ca2+ extrusion via Na/Ca exchange, there were no differences in pause-dependent potentiation between the atrial and ventricular myocardium. This may suggest that Na/Ca exchange plays an important role in the reversal of rest potentiation.

Differences in SR Ca2+ handling in atrial and ventricular myocardium. The oxalate-supported Ca2+ uptake rate in tissue homogenate, which reflects the intrinsic Ca2+ transport by SR Ca2+-ATPase (26), was taken to estimate the SR function in vitro. Our data indicate that the oxalate-supported Ca2+ uptake rate (expressed per milligram of protein) is two times higher in atria than in ventricles. This may be partly explained by the 30% higher level of the SERCA2 mRNA relative to 18S RNA observed in atria (Fig. 7) and by the lower expression of phospholamban (17), favoring a more efficient Ca2+ uptake rate in atria than in ventricles. The similar free Ca2+ concentration that produces half-maximal activation of the SR Ca2+ pump in atria and ventricles is in agreement with the observation of an unchanged SERCA2a-to-SERCA2b ratio.

To evaluate the interaction of SR with the contractile apparatus, the SR function was analyzed in situ, in saponin-permeabilized fibers. In both atria and ventricles, caffeine-induced Ca2+ release was observed to occur in two phases. The fast phase of Ca2+ release was evidenced by T/Tmax which saturated faster and appeared lower in atria than in ventricles. This smaller
fast phase of Ca\textsuperscript{2+} release might be the basis for the lower DT observed in atria. However, a further increase in the loading time induced a prolongation of the tension transient as evidenced by the progressive increase in the ST. This prolongation of the tension transient was more marked in atria than in ventricles. The time course of the caffeine-induced tension transient will depend on 1) the amount of released Ca\textsuperscript{2+}, 2) the rate of SR Ca\textsuperscript{2+} release, 3) the diffusion of Ca\textsuperscript{2+} away from the myofibrils, 4) the Ca\textsuperscript{2+} buffering by EGTA and proteins, 5) the possible reuptake and release of Ca\textsuperscript{2+} and, 6) the Ca\textsuperscript{2+}-sensitizing effect of caffeine. Because atrial and ventricular fibers have similar diameter and composition, it is unlikely that differences will arise from different Ca\textsuperscript{2+} buffering. Similarly, atrial and ventricular fibers exhibited the same sensitivity to Ca\textsuperscript{2+} (31) and similar sensitivity to Ca\textsuperscript{2+} in the presence of caffeine (this study), allowing the comparison of force transients. Moreover, increasing caffeine concentration or reapplying caffeine quickly after the first application did not modify atrioventricular differences, suggesting that these differences did not arise from incomplete Ca\textsuperscript{2+} release or differences in caffeine sensitivity. The appearance of a second slow phase of tension could be considered as a result of prolonged Ca\textsuperscript{2+} release, suggesting different mechanisms of Ca\textsuperscript{2+} release in atria and ventricles. On the other hand, the similar tension-time integrals suggested that the amount of releasable Ca\textsuperscript{2+} appeared to be the same in the two tissues, whatever the speed of Ca\textsuperscript{2+} release or the duration of SR loading. These results, together with the similar amount of mRNA for Cals, the main Ca\textsuperscript{2+}-buffering protein in SR, suggest that the capacity of the SR Ca\textsuperscript{2+} pool is similar in atrial and ventricular myocardium.

Slow SR Ca\textsuperscript{2+} release in atria appeared not to be due to a lower amount of SR Ca\textsuperscript{2+} channel, because the same degree of expression of RyR mRNA suggested a similar amount of Ca\textsuperscript{2+} release channels in both tissues. An alternative explanation may be morphological differences between atria and ventricles. In human atrial cells, it was shown that the intracellular Ca\textsuperscript{2+} transient triggered by membrane depolarization is not entirely controlled by the Ca\textsuperscript{2+} current and results from the activation of two components of Ca\textsuperscript{2+} signals (14). Berlin (8), using confocal microscopy, showed that, in guinea pig atrial myocytes devoid of t tubules, stimulated increases in internal Ca\textsuperscript{2+} can be observed to arise
in focal regions of the cell before spreading to the cell interior. In intact rabbit ventricular cardiomyocytes, Bassani et al. (7) showed that one-half of the Ca$^{2+}$ in the SR is released during a twitch, whereas the beat-dependent depletion of SR Ca$^{2+}$ is biexponential. They suggest that the slower phase might represent a caffeine-sensitive pool of Ca$^{2+}$ not normally released during a twitch and speculated that the Ca$^{2+}$ in the corbular SR could represent such a pool. In comparison with ventricular cardiomyocytes, atrial cells are characterized by the absence of a tubular system (23). They contain only peripheral junctional SR connected to sarcocremma and a higher proportion of corbular SR within the cytoplasm. Because corbular SR is not connected to sarcocremmal membrane, Ca$^{2+}$ release from these stores must be triggered by a diffusible agent (15). The delayed phase of Ca$^{2+}$ release in atria could thus reflect Ca$^{2+}$ released from these internal cisternae by a slower Ca$^{2+}$-induced Ca$^{2+}$-release mechanism.

CK and SR Ca$^{2+}$ uptake in atria. The rapid work of the SR Ca$^{2+}$ pump in atria critically depends on adequate energy supply and effective withdrawal of ATPase reaction products. The isolated SR membranes of several muscle types have been shown to contain strongly anchored MM-CK that is functionally coupled to the Ca$^{2+}$ pump (32). Recently, we have confirmed the coupling between bound CK and SR Ca$^{2+}$-ATPase in situ in saponin-skinned ventricular fibers with preserved local architecture by showing that localized regeneration of ATP at the expense of PCr and ADP is more efficient to meet the requirement of Ca$^{2+}$-ATPase than external ATP (24). The role of CK in providing energy for the SR Ca$^{2+}$ pump in situ could be demonstrated by efficient loading of the SR in the presence of PCr and ADP. These results showed that local ATP generated by the CK reaction was sufficient to completely meet the ATP requirements of the SR Ca$^{2+}$-ATPase in atria. In contrast, ATP alone was not able to sustain control loading of the SR. Our study clearly demonstrates that, in atria, the CK system bound to structures at sites of energy utilization is at least as efficient as in ventricles to provide energy for SR Ca$^{2+}$ uptake and to maintain a favorable local ATP-to-ADP ratio in the vicinity of the ATPase.

In conclusion, fast and efficient CK-supported SR Ca$^{2+}$ uptake together with limited or slow Ca$^{2+}$ release and diffusion for contraction could be proposed as the main modulator of the shorter time course and lower tension development in rat intact atrial myocardium compared with that in ventricular myocardium, despite a similar total capacity of releasable Ca$^{2+}$ in both tissues.

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