Functional Expression of the TRPM4 Cation Current in Ventricular Cardiomyocytes From Spontaneously Hypertensive Rats

Romain Guinamard, Marie Demion, Christophe Magaud, Daniel Potreau, Patrick Bois

Abstract—Cardiac hypertrophy is associated with electrophysiological modifications, including modification of action potential shape that can give rise to arrhythmias. We report here a higher detection of a calcium-activated nonselective cation current in spontaneously hypertensive rats (SHRs), a model of hypertension and heart hypertrophy when compared with Wistar–Kyoto (WKY) rat, its normotensive equivalent. Freshly isolated cells from the left ventricles of 3- to 6-month-old WKY rats or SHRs were used for patch-clamp recordings. In inside-out patches, the channel presented a linear conductance of 25 ± 0.5 pS, did not discriminate Na⁺ over K⁺, and was not permeable to Ca²⁺. Open probability was increased by depolarization and a rise in [Ca²⁺], (dissociation constant = 10 ± 5.4 μmol/L) but reduced by 0.5 mmol/L [ATP], 10 μmol/L glibenclamide, or flufenamic acid (IC₅₀ = 5.5 ± 1.7 μmol/L). Thus, it owns the fingerprint of the TRPM4 current. Although rarely detected in WKY cardiomyocytes, the current was present in >50% of patches from SHR cardiomyocytes. Moreover, by performing RT-PCR from ventricular samples, we observed that TRPM4 mRNA detection was higher in SHRs than in WKY rats. We propose that a TRPM4 current is expressed in ventricular cardiomyocytes from SHRs. According to its properties, this channel may contribute to the transient inward current implicated in delayed-after-depolarizations observed during [Ca²⁺] overload of cardiomyocytes. (Hypertension. 2006;48:587-594.)

Key Words: hypertrophy ■ cation channel ■ ventricular function

Ventricular arrhythmias occur frequently in patients with cardiac hypertrophy before progression to heart failure. Studies performed using animal models of cardiac hypertrophy have provided a better understanding of the electrophysiological alterations accompanying this condition and of the mechanisms leading to arrhythmias.¹

The spontaneously hypertensive rat (SHR) is a well-established genetic model of hypertension and cardiac hypertrophy when compared with its normotensive equivalent, the Wistar–Kyoto rat (WKY). The chronic hypertension gives rise to heart failure. Thus, because cardiac hypertrophy in humans is often associated with chronic hypertension, the SHR is a realistic model of human hypertrophy.¹ Structural characteristics of hypertrophy have been detected in whole SHR heart and in single myocytes from the left ventricle.²³ During the compensated stage (from 3 months of age), an increase in myocyte contractility, amplitude of the intracellular Ca²⁺ transient, and action potential duration (APD) have been observed, with no changes in resting membrane potential.⁴⁵ The APD prolongation is correlated with a prolonged QT interval on the ECG⁶ and is mainly attributable to a reduction of the transient outward current (Iₒ) density.⁷ Moreover, SHR ventricular myocytes exhibit an inward current having the same properties of the pacemaker current If⁸⁹ that would favor the appearance of ventricular arrhythmias.

In addition to the above-mentioned changes, Ruocco et al¹⁰ reported a higher incidence of delayed-after-depolarizations (DADs) and associated-after-contractions in SHR ventricular myocytes compared with WKY rats. DADs are generated by intracellular Ca²⁺ waves caused by a spontaneous and local Ca²⁺ release from intracellular stores. In accordance with this mechanism, the incidence of cells presenting calcium waves is greater in cardiomyocytes isolated from SHR.¹¹ Thus, any modification of Ca²⁺-activated currents could participate in these DADs. Interestingly, we observed previously a [Ca²⁺]-activated nonselective cation channel (NSC₉⁻) in normotensive rat ventricular myocytes in primary culture; these cells undergo a dedifferentiating process with properties similar to that which occurs in hypertrophied cells.¹²¹³ Channel detection was found to increase with degree of dedifferentiation. The channel shares electrophysiological properties with the TRPM4 channel protein.¹⁴¹⁷

The purpose of the present experiments was to determine the incidence of such an NSC₉⁻ channel in left ventricular...
myocytes from SHRs in comparison with normotensive WKY rats. We report that the functional current is more detected in hypertrophied myocytes from SHR, as also is TRPM4 mRNA.

**Methods**

**Cell Isolation**

Experiments were performed in accordance with European Community Council directives. Rat ventricular myocytes were obtained as described previously. Adult (3 to 6 months) male SHRs and WKY rats were injected with 1000 IU of heparin and 0.3 g/kg of hydroxycholplor (Choay, Nanofi). The heart was mounted on a Langendorff apparatus and perfused at (37°C) with the following oxygenated solutions: 4 minutes with Tyrode solution; 4 minutes with a CaCl2-free Tyrode solution, and 15 to 20 minutes with the latter solution supplemented with 100 IU/mL collagenase (type II, Worthington), 0.06 mmol/L CaCl2, and 0.1% BSA. Once flaccid, the heart was rinsed with the same solution without collagenase. The left ventricle was removed, immersed in Kraft–Bru¨he medium, and ventricle was removed, immersed in Kraft–Bru¨he medium, and maintained at 37°C in a moist environment. Cells were seeded onto 35-mm Petri dishes pretreated with a Ca2+ buffer and bath solution contained (in mM): 140 NaCl; 4.8 KCl; 1.2 MgCl2; 10 glucose; and 10 HEPES. Pipette and bath solutions were adjusted to pH 7.4 with NaOH. The pH of solutions and Chemicals

**Data Analysis**

Signals were analyzed with Bio-patch software (version 3.30; Biologic). Amplitude histograms were generated to construct I/V curves and estimate open probability (Po). Relative permeabilities and associated reversal potentials for current flow (Vrev) were estimated by fitting experimental measurements to the Goldman–Hodgkin–Katz equation. Po as a function of voltage was fitted using a Boltzmann function: $P_o = P_{max} \times (P_{min} - P_{max})/(1 + exp(V - V_{50})/s)$, where $P_{min}$ and $P_{max}$ represent the minimum and maximum open probabilities, respectively, $V_{50}$ is the potential for half maximal activation, and $s$ is the slope parameter. Experimental results are reported as mean±SEM. A paired Student t test was applied when $P_o$ was determined under different conditions on the same cell. Statistical significance was accepted for values of $P<0.05$. A Fisher’s exact test was used to compare the percentage of patches with channel activity under different conditions.

**ECGs**

ECGs were recorded using the power laboratory 4/20 interface (ADInstruments) and chart5 software (ADInstruments) from anesthetized animals. QT and RR durations were measured manually by evaluating the QT duration as the time elapsed between the onset of the Q wave and the end of the QT complex. Calculations were made from the average of 10 RR and QT complexes. Considering QT variations related to frequency, the QT was corrected according to the equation QTC = QT/(RR×100)12 suitable for small animals (QT and RR in milliseconds).20

**Patch-Clamp Measurements**

Single-channel currents were recorded from membrane patches of isolated cardiomycocytes held under voltage clamp with a RK400 (Biologic) patch-clamp amplifier, using the inside-out configuration of the patch-clamp technique. The bath reference was 0.5 mol/L KCl (in a 4% agar bridge) connected to an Ag/AgCl pellet. Liquid junction potentials arising from changes in bath solutions at the inner surface of the membrane patch were determined using a pipette containing 2.7 mol/L KCl. Applied potentials ($V_{pipet}=V_{hold}−V_{pipet}$) were corrected accordingly. Experiments were conducted at room temperature (20°C to 25°C).

**Solutions and Chemicals**

For cell dissociation, the Tyrode solution contained (in mM): 140 NaCl; 5.4 KCl; 1.8 MgCl2; 1.8 CaCl2; 10 glucose; and 10 HEPES; pH was adjusted to 7.35 with NaOH. For patch clamp experiments, the standard 140 mmol/L NaCl solution for bath and pipette contained (in mM): 140 NaCl; 4.8 KCl; 1.2 MgCl2; 10 glucose; and 10 HEPES. Pipette and bath solutions contained 1 mmol/L and 1.8 mmol/L of CaCl2, respectively. When specified, [Ca2+]o <10 μmol/L was determined with a combination of CaCl2, and Ca-EGTA buffers or addition of EGTA. Low NaCl solutions contained 14 or 42 mmol/L of NaCl (no KCl included) and were supplemented with sucrose to maintain osmolarity. To test monovalent cation selectivity, 140 mmol/L of NaCl were replaced by KCl. KCl permeability was determined using a solution containing (in mM): 100 CaCl2; 10 mM glucose; and 10 HEPES. External solutions (pipette and bath) were adjusted to pH 7.4 with NaOH. The pH of perfused solutions was adjusted to 7.2. The effect of internal ATP on channel activity. Chemicals were from Sigma.

**RT-PCR Analysis**

Left ventricular and atrial samples were collected from WKY rats and SHRs. Total RNA was extracted by the Chomczynski method, using the RNAble reagent (Eurobio). cDNA was synthesized with the Pd(N), random hexamer primer (Amersham). Ten microliters of RNA, 2.4 μg of random primers, 11.3 mmol/L of DTT (Gibco), and 1.1 mmol/L of dNTPs (Invitrogen) were mixed in a total volume of 22 μL and incubated for 2 minutes at 65°C. Then, 30 U of RNAguard (Amersham) and 44 U of reverse transcriptase M-Murine leukemia virus (Invitrogen) were added and incubated for 1 hour at 37°C, in a final volume of 50 mL. Primers for amplification were TGGCATACTGGAGACCGCA and GGCCCAAGATCGCTCATCGT for TRPM4, GGAGAGACCTGGCTCGACCC and TCGAACACACGTTTTTGA for TRPM5, and TATCTTTCATTGGTG and AATTGAAAGCGGAGTCACAG for 18S RNA. The PCR amplification was carried out with 0.5 to 1 μg of cDNA, 0.25 mmol/L of dNTPs (Invitrogen), 0.4 μmol/L of each primer, 2 mmol/L of MgCl2, 1.25 U of Taq polymerase (Gibco), and 1X Taq buffer (Gibco) in a total volume of 25 μL. Thermal cycles performed in a PTC-100 (M.J. Research, Inc) were: 95°C for 3 minutes, followed by 30 cycles at 59°C for 30 s, 72°C for 30 s, and 95°C for 30 s. Amplification was stopped by a step at 59°C for 2 minutes and at 72°C for 10 minutes. Amplified products were electrophoresed on 1% agarose gels and stained with ethidium bromide. They were then sequenced using the ABI prism Big Dye terminator cycle method (Applied Biosystems, Perkin-Elmer). Optical density ratios were determined using the Photoshop software by comparing density for TRPM4 to 18S RNA.
Results

Morphological and Electrophysiological Modifications in SHRs

SHR animals had significantly elevated whole heart/body weight ratios compared with WKY rats, with an increase of 55.5% (Figure 1A). Also, myocyte surface area was significantly increased by 67.3% and cell capacitance by 50.6% in SHRs compared with WKY rats (Figure 1B and 1C). These data indicate, as expected, heart hypertrophy in SHRs. Micrograph images of typical myocytes from SHRs and WKY rats are presented in Figure 1D.

Electrocardiograms from SHRs and WKY rats were performed (Figure 1E). The QT interval corrected value QTc was significantly increased by 21.7% in SHR compared with WKY animals (Figure 1E). This indicates that the SHR used here developed classical electrophysiological perturbations associated with ventricular APD prolongation.

Channel Properties in SHRs

We reported previously the expression of a 25 pS NSCCa channel in WKY rat ventricular myocytes in culture that undergo a dedifferentiating process with properties similar to hypertrophied cells. Here we investigated its functional expression in cardiomyocytes isolated from the SHR left ventricle.

Channel activity was recorded in the inside-out patch configuration, using 140 mmol/L NaCl standard solution on both sides.
of the membrane ([CaCl2]i=1.8 mmol/L; [Cl]i=1 mmol/L), called control condition. Single channel currents were detected in some patches. Figure 2A illustrates typical channel activity. The corresponding current-voltage relationship (Figure 2B) was linear with a slope conductance of 25±0.6 pS and reversal potential (Vrev) estimated to be 0.1±2.5 mV. P0 values determined for various membrane potentials increased with depolarization (Figure 2C). Data were fitted to a Boltzmann function with the parameters 0.98±0.02 for P0, 19.3±2.7 mV for s, and +27.8±2.7 mV for Vrev (n=4).

Ion selectivity was assessed by ion substitution experiments (see Table). When a patch was exposed to 140 mmol/L of NaCl in the pipette (extracellular side) and 42 or 14 mmol/L NaCl in the bath (intracellular side), the current–voltage relationship shifted toward the positive voltage range (Figure 2D) indicating a cationic selectivity. Replacement of NaCl (140 mmol/L) in the bath solution by KCl (140 mmol/L) did not significantly affect channel conductance or Vrev, indicating that the channel does not discriminate K+ over Na+.

Channel Regulation in SHRs

As has been reported for several tissues23 in addition to human and rat cardiomyocytes,12-22 the nonselective cation channel in SHR myocytes is also dependent on [Ca2+]i, and internal ATP. The P0 of active channels at +40 mV is plotted in Figure 2E as a function of [Ca2+]i rising from 1 mol/L to 1 mmol/L (n=4). Channel openings were seen at [Ca2+]i≥0.1 μmol/L. Fitting of the Hill equation (see Figure 2 legend) to the experimental data points yielded an apparent dissociation constant (Kd) of 10±5.4 μmol/L and a Hill coefficient of 0.6±0.1.

Addition of 5.10−4 M ATP (corresponding with 40 μmol/L free-ATP) to the cytoplasmic side reversibly decreased P0 to 22±8% of control (Figure 3B; Vm=+40 mV; n=6). The effect caused by ATP could be indicative of a sulfonylurea sensitivity, in a manner analogous to the nonselective cation channel from native reactive astrocytes24 or human atrial cardiomyocytes.22 On this basis, we assessed the effect of the sulfonylurea glibenclamide, a hypoglycemic agent, on SHR cardiomyocytes. Figure 3A and 3B illustrates the reversible action of 10 μmol/L of glibenclamide on channel activity.

### Ionic Selectivity

<table>
<thead>
<tr>
<th>Bath Solution</th>
<th>Conductance, pS</th>
<th>Vm, mV</th>
<th>Permeability Ratio</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>140 NaCl</td>
<td>25±0.6</td>
<td>+0.1±2.5</td>
<td>P0/P0u=0.11</td>
<td>4</td>
</tr>
<tr>
<td>42 NaCl</td>
<td>24.5±1.8</td>
<td>+23.3±1.2</td>
<td>P0/P0u=0.11</td>
<td>3</td>
</tr>
<tr>
<td>14 NaCl</td>
<td>26.5±2</td>
<td>+38.3±2</td>
<td>P0/P0u=0.11</td>
<td>4</td>
</tr>
<tr>
<td>140 KCl</td>
<td>24±2.6</td>
<td>−0.4±1</td>
<td>P0/P0u=0.98</td>
<td>4</td>
</tr>
<tr>
<td>100 CaCl2</td>
<td>26±4.0</td>
<td>+43.3±6.7</td>
<td>P0/P0u=0.12</td>
<td>4</td>
</tr>
</tbody>
</table>

Current-voltage relationships were determined with the bath containing the solution as indicated. Pipettes were filled with the 140 mmol/L NaCl solution. Relative permeabilities (P0/P0u) were derived from the mean reversal potential (Vrev) using the Goldman–Hodgkin–Katz equation.22
The drug reduced channel activity to $22.7 \pm 12.5\%$ of that of control ($V_n = +40$ mV; $n = 4$).

Flufenamic acid, a nonsteroidal anti-inflammatory drug, blocks nonselective cation channels$^{12,22,25}$ and is a useful agent for distinguishing between TRPM4 and TRPM5 currents.$^{17}$ Figure 3C illustrates the reversible action of this compound on channel activity. Channel inhibition was complete at 100 $\mu$mol/L. The dose–response curve for flufenamic acid is shown in Figure 3D, where the activity as a percentage of the control is plotted against the blocker concentration. Half-maximal inhibition by flufenamic acid was achieved at $5.5 \pm 1.7$ $\mu$mol/L.

As reported previously for nonselective cation channels characterized from cardiac$^{12,22}$ and cerebral artery smooth muscle cells,$^{26}$ channel detection was increased significantly after stimulation of the protein kinase C (PKC) pathway. In myocytes dissociated from SHR hearts, channel activity was rarely detected after preincubation of the myocytes in the control 140 NaCl external solution (4 of 33 cells). However, when cells are incubated for $\geq 10$ minutes, before establishment of the patch, in the presence of the ATP analogue ATP$_7$S (100 $\mu$mol/L), a purinergic receptor agonist activating the phospholipase C cascade, channel activity was detected in 26 of 50 patches. This increase in channel detection rate was abolished if cells were incubated before patching with 100 $\mu$mol/L ATP$_7$S in the presence of 1 $\mu$mol/L calphostin C, a specific blocker of PKC (4 of 27 cells). To confirm the implication of the PKC pathway in channel activation, cells were incubated with the PKC activator PMA (0.5 $\mu$mol/L). Data summarized in Figure 3E indicate an increase in channel detection (46 of 78 patches).

### Channel Detection in WKY and SHR Rats

To evaluate the effect of chronic hypertension on the functional expression of the NSC$_{Ca}$ channel, detection of the channel was also investigated in freshly isolated cardiomyocytes from normotensive rats. Results indicate that no activity was detected under control conditions (0 of 25) and that only a few patches showed channel activity after preincubation with either ATP$_7$S or PMA (1 of 28 and 3 of 41 cells, respectively; not statistically different from control). These data match with previous reports describing only a low detection rate or absence of channel activity in freshly isolated ventricular cells from normotensive rats$^{12,13}$ or guinea pigs.$^{27}$ The mean number of active channels per patch was calculated and called channel density. As reported in Figure 4A, channel density was significantly higher in SHRs compared with WKY in PMA-treated cells.

To eliminate the hypothesis that the increase in channel density arises from a genetic disparity, channel density was evaluated in the function of cardiac hypertrophy in SHRs. Animals were pooled by age when cardiac hypertrophy develops (3 to 5 animals for each batch), then mean heart/body ratio and channel density after PMA treatment were calculated. A significant positive correlation was found between cardiac hypertrophy and channel density (Figure 4B).

### TRPM4 and TRPM5 mRNA Expression in Ventricular Cells

The cardiac NSC$_{Ca}$ channel described here shares biophysical properties with the TRPM4 channel.$^{14}$ RT-PCR was used to probe for TRPM4 in WKY and SHR cardiac tissues. Atrial and left ventricular samples were used. In accordance with our previous report on human atrial cells,$^{22}$ TRPM4 messen-
ger was detected in the atrium from both WKY rats and SHRs. At the ventricular level, TRPM4 messenger was only weakly detected in WKY rats. The mean optical density significantly (TRPM4/18S) increased by 5-fold in SHRs compare with WKY rats. Figure 4C and 4D shows an example of amplification and the mean optical density ratio histogram. Amplified products were sequenced and were similar (98%) to the predicted rat TRPM4 mRNA sequence (MN 133607).

We also preformed RT-PCRs to probe for TRPM5, which is structurally and functionally close to TRPM4. A significant level of amplified product was detected in both WKY and SHR ventricle, but no significant variation was observed related to hypertrophy (density ratios TRPM5/18S: 0.78±0.12 in WKY, n=4, and 0.5±0.15 in SHR, n=3).

Discussion

A TRPM4-Like Current

We show here that an NSCC\textsubscript{Ca} is present in SHR ventricular cardiomyocytes. It exhibits the classic characteristics of NSCC\textsubscript{Ca} described for a large number of tissues. These characteristics include voltage dependency, conductance around 25 pS, no discrimination between monovalent cations, no or very weak permeability to calcium, sensitivity to intracellular calcium, inhibition by cytosolic ATP, and blockade by flufenamic acid.\textsuperscript{23} Also, channel detection was increased by stimulation of the PKC pathway, using ATP\textsubscript{PyS} or PMA. This increase was abolished in the presence of the PKC inhibitor calphostin C. We reported previously the presence of such a channel in human atrial cardiomyocytes and dedifferentiated cardiomyocytes from the WKY rat ventricle.\textsuperscript{12,22} Values for single channel conductance, ionic permeabilities, and dependence on calcium and PKC were similar to those observed here in SHRs.

The recent discovery of the transient receptor potential (TRP) protein family has revived interest in nonselective cation channels. Within the TRPM subfamily,\textsuperscript{28} TRPM4 generates a current similar to that observed in SHR ventricular cardiomyocytes. TRPM4 acts as a nonselective cation channel of 25 pS that is activated by [Ca\textsuperscript{2+}]\textsubscript{i}, with a \(K_d\) ranging between 0.4\textsuperscript{14} and 9.8 \(\mu\text{mol/L}^{29}\). Its voltage dependence follows a Boltzmann-type relationship with a \(V_{0.5}\) of 9.7 mV. It is equally selective for Na\textsuperscript{+} and K\textsuperscript{+}\textsuperscript{14} and is blocked by intracellular free-ATP in the 10 \(\mu\text{mol/L}\) range.\textsuperscript{28} In addition, activation of the TRPM4 current by PKC was also reported when expressed in HEK293 cells.\textsuperscript{50}

The cardiac NSCC\textsubscript{Ca} might also be generated by TRPM5, which acts as a 25-pS voltage and calcium-sensitive nonselective cation channel.\textsuperscript{31} However, TRPM5 is not inhibited by ATP.\textsuperscript{17} Also, TRPM4 has a higher sensitivity to flufenamic acid than TRPM5, with estimated IC\textsubscript{50} values of 2.8 and 24.5 \(\mu\text{mol/L}\), respectively.\textsuperscript{17} The cardiac NSCC\textsubscript{Ca} IC\textsubscript{50} value of 5.5 \(\mu\text{mol/L}\) is closer to that of TRPM4. Finally, a third difference to be highlighted is the presence of an ABC signature motif in TRPM4 that is absent in TRPM5.\textsuperscript{17} Inhibition of the cardiac NSCC\textsubscript{Ca} by glibenclamide indicates possible similarities with the cystic fibrosis transmembrane conductance regulator and sulfonamide receptors that have the ABC signature. Taken together, the cardiac NSCC\textsubscript{Ca} properties match the TRPM4 fingerprint almost perfectly, showing that TRPM4 is the molecular correlate of this channel.

Current Detection and Hypertrophy

The major finding arising from the present study is the detection of an increase in the TRPM4 current activity in myocytes from SHRs compare with WKY rats. Indeed, channel activity was rarely observed in freshly isolated cardiomyocytes from normotensive rats, even after PKC stimulation. On the contrary, in SHRs, it was recorded in 50% of patches in such conditions. This altered activity could be because of an increase in TRPM4 protein expression or an increase in a factor such as PKC that alters its activity. Both are probably implicated. First, it was reported that diacyl-glycerol content and PKC activity increase during cardiac hypertrophy.\textsuperscript{32,33} Second, the rise in TRPM4 mRNA expression reported here is in favor of an increase in the amount of proteins.
The activation properties of TRPM4 predict a complex physiological implication. Both depolarizing phases and increases of internal calcium will favor its activation. Then, according to its equal selectivity for Na⁺ and K⁺, it will produce a combination between an inward depolarizing Na⁺ current and an outward repolarizing K⁺ current. Calcium waves appear in SHR myocytes during the development of hypertrophy, which, in turn, induce DADs. DADs are because of the development of a calcium-dependent current, called the transient inward current (Iₒ). Iₒ has been extensively studied in cardiac cells, and is assumed to be composed of 3 components; the Na⁺/Ca²⁺ exchanger, a [Ca²⁺]-activated chloride current, and a [Ca²⁺]-activated nonselective cation current. The channels responsible for this third component have not been described at the unitary level. A 30 to 40 pS calcium-activated nonselective channel has been described in neonate rat myocytes; however, its presence was not reported in adult cells. The present description of TRPM4 in SHRs provides another candidate for this current, because intracellular calcium is also one of its major regulators. Interestingly, in human cardiomyocytes, a calcium-activated nonselective cation current at the whole-cell level was reported to be implicated in Iₒ. This whole-cell calcium-activated nonselective cation current was detected in atria but not in ventricles. That is in accordance with our results. Indeed, in human atria, we detected TRPM4 single channels currents but no currents or weak currents in normotensive rat ventricles. Also, in those rats, TRPM4 mRNA was higher in atria than ventricles. Thus, we hypothesize that TRPM4 could participate in Iₒ, in atrial but not ventricular normotensive rat cardiomyocytes. On the other hand, it could contribute to this current in SHR ventricular cells and, thus, participate in the increase in DAD occurrences in those rats.

Also, TRPM4 could participate in the proarrhythmic early after depolarizations (EADs) during the cardiac action potential. Indeed, EADs are associated with secondary Ca²⁺ release of the sarcoplasmatic reticulum. In addition, because the cell is depolarized by the time of action potential, TRPM4 is in its voltage range of activation. By producing its nonselective cation current, it would participate to the perpetuation of EADs. Although no data are available in SHRs, it has to be noted that ventricular hypertrophy induces EADs in a rabbit heart. Moreover, as Ca²⁺ transient that is increased in SHR modifies APD by modulating Ca²⁺-sensitive channels, the presence of TRPM4 in SHRs could participate to the increase of APD reported in SHRs.

Because its biophysical and regulatory properties close to TRPM4, one can imagine that TRPM5 could also participate in these Ca²⁺-induced mechanisms. However, whereas we detected TRPM5 mRNA in ventricles, we did not identify the channel at the functional level. Also, its expression was not modified in SHRs.

In conclusion, we infer that the TRPM4 current is expressed in SHR ventricular myocytes and, because of its electrophysiological properties, it could participate in several processes of cardiac arrhythmias associated with hypertrophy.

**Perspectives**

We showed the presence of a TRPM4 current in SHR cardiomyocytes and infer that it could, among other ionic components, participate in proarrhythmic processes related to cardiac hypertrophy. The development of specific pharmacological tools for TRPM4 would permit the determination of its contribution to the ventricular cardiac action potential and its modifications induced by hypertrophy. Also, production of TRPM4 knockout mice will be a step forward in elucidating its function in cardiac electrical activity. The knowledge of this current should help in the understanding of the action of pharmacological compounds used in the modulation of cardiac activity and then sharpen their action.

**Acknowledgments**

We thank Dr Anne Cantereau for assistance with microscopy and Chantal Jouglà, Françoise Mazin, and Sylvain Normand for technical help.

**Sources of Funding**

This study was supported by funding from the French Centre National de la Recherche Scientifique and the University of Pottiers. M.D. holds a PhD fellowship from the French Ministère de l’Enseignement et de la Recherche.

**Disclosures**

None.

**References**


Functional Expression of the TRPM4 Cationic Current in Ventricular Cardiomyocytes From Spontaneously Hypertensive Rats
Romain Guinamard, Marie Demion, Christophe Magaud, Daniel Potreau and Patrick Bois

Hypertension. 2006;48:587-594; originally published online September 11, 2006; doi: 10.1161/01.HYP.0000237864.65019.a5
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/48/4/587

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/