Hyperpolarization-Activated Inward Current in Ventricular Myocytes From Normal and Failing Human Hearts

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Background—The hyperpolarization-activated inward current (If) was found to be overexpressed in hypertrophied rat ventricular myocytes, indicating that If might favor arrhythmias in hypertrophied or failing ventricular myocardium. In the present study, we evaluated whether If is expressed in human ventricular myocardium, if it may be increased in human heart failure, and if its autonomic modulation may be altered.

Methods and Results—The whole-cell patch-clamp technique was used to record If in isolated ventricular myocytes from 34 failing (dilated [DCM] or ischemic [ICM] cardiomyopathy) and 13 donor hearts (NF). If was observed in all myocytes showing typical current properties, ie, time and voltage dependence, block by [Cs+]o, permeability for K+ and Na+, and current increase with raising [K+]o. There was a trend toward larger current densities in myopathic (at −130 mV in [K+]o, 25 mmol/L; DCM: −1.37 ± 0.12 pA/pF, n = 50; ICM: −1.39 ± 0.24 pA/pF, n = 30) than in nonfailing cells (−1.18 ± 0.21 pA/pF, n = 24), although this difference did not reach statistical significance (P = .23). Boltzmann distributions yielded an activation threshold of −80 mV and half-maximal activation at −110.96 ± 0.06 mV in myopathic and normal myocytes. Isoproterenol (10−5 mol/L) shifted the current activation by 10 mV (31 myopathic, 5 NF). Carbachol and adenosine had no direct effect on If (6 and 12 myopathic, 3 and 3 NF, respectively) but reversibly antagonized β-adrenergic stimulation (5 and 7 myopathic, 2 and 2 NF, respectively). Autonomic modulation was similar in failing and nonfailing cells.

Conclusions—In end-stage heart failure, no significant change of If could be found, although there was a trend toward increased If. Together with an elevated plasma norepinephrine concentration and a previously reported reduction in IK1 in human heart failure, If might favor diastolic depolarization in individual myopathic cells. (Circulation. 1998;97:55-65.)

Key Words: electrophysiology • heart failure • ventricles • adenosine • arrhythmia

Congestive heart failure is a common and highly lethal cardiovascular disorder, with an annual mortality as high as 50%–6. From 35% to 50% of these death are sudden and unexpected.7–9 Most sudden cardiac deaths in heart failure are measured in multicellular and in single-cell recordings.16–18 However, mechanisms in severe heart failure has focused mainly on alterations of repolarization.19–24 In failing human hearts, a reduction in the transient outward current (Iₒ) and of the inward rectifier current (IK1) have been demonstrated,16 consistent with an action potential prolongation measured in multicellular and in single-cell recordings.16–18 However, spontaneous diastolic depolarizations may also initiate arrhythmias in diseased myocardium.

In sinus node and Purkinje cells, the hyperpolarization-activated inward current (If) is considered to contribute significantly to the spontaneous diastolic depolarization phase.19–24 If is a nonselective cation inward current that is blocked by extracellular cesium.21–25 If was found to be stimulated by β-adrenoceptor agonists through a shift of the current activation curve to more positive potentials.21–22,25 An If-like current has also been recorded in ventricular myocytes of mammalian species, such as guinea pigs,26 dogs,25,26 and rats.27,28 In spontaneously hypertensive rats, If density was linearly related to the severity of cardiac hypertrophy and was found to be significantly larger than in undiseased control animals.28 This led to the hypothesis that overexpression of If might contribute to the increased propensity of arrhythmias in hypertrophied ventricular myocardium.28 More recently, we (published in abstract form)26 and Cerbai et al29 recorded a hyperpolarization-activated inward current with properties similar to If in isolated human ventricular myocytes. Our preliminary data suggested an increased If density in end-stage heart failure compared with cells from undiseased control hearts.29 Cerbai et al30 investigated myocytes from three failing hearts but not from nonfailing controls. Therefore, the aim of the present study was to investigate whether If is overexpressed in ventricular myocytes isolated from...
hearts of patients with terminal heart failure. Furthermore, the effects of β-adrenergic-, muscarinic-, and A1-receptor-mediated stimulation, which might additionally increase or decrease If, have not yet been evaluated in human myocytes. The possible functional relevance of If for the initiation of arrhythmias in human heart failure is also discussed.

**Methods**

**Patients**

Ventricular myocytes were isolated from 34 hearts of patients with end-stage heart failure caused by dilated cardiomyopathy (n=23) or ischemic cardiomyopathy (n=11) undergoing transplantation. All patients received digoxin and diuretics. No catecholamines or β-adrenoceptor blocking drugs were given during 48 hours before transplantation. Informed consent was obtained before organ transplantation. Results were compared with cells prepared from 13 human hearts without heart failure that could not be transplanted for technical reasons (coronary artery disease without myocardial infarction or heart failure [n=10]; possible systemic infectious disease [n=2]; and blood group incompatibility [n=1]). The isolation procedure was identical in all hearts used.

**Cell Isolation**

The isolation procedure was described in detail before. A part of the left ventricular wall was excised, together with its arterial branch. The wall segment was then perfused via its arterial branch: 30 minutes with nominally Ca2+-free modified Tyrode’s solution ([mmol/L] NaCl 135, KCl 4, MgCl₂ 1, glucose 10, NaH₂PO₄ 0.33, and HEPES-NaOH 10; pH was adjusted to 7.3 with the addition of NaOH, 37°C), followed by 40 minutes with the same solution with added collagenase (type II, 200 IU/mL; Worthington) and protease (type XIV, 0.3 IU/mL; Sigma Chemical Co). Finally, the enzyme was washed out for 15 minutes with modified Tyrode’s solution that contained 100 μmol/L CaCl₂. Cells used in this study were taken from the central part of the myocardial wall. Cells were disaggregated by mechanical agitation and, after filtering through a nylon mesh, were stored at room temperature in Tyrode’s solution containing 2.0 mmol/L CaCl₂.

The living-cell yield was 5% to 10%. Only cells with clear striation without significant granularity were selected for experiments. In the Tyrode’s solution that was used to store cells (containing 4 mmol/L KCl, 2 mmol/L CaCl₂), we observed few cells with slow spontaneous contractions (~20 to 30 oscillations per minute). By face value, the frequency and number of spontaneously beating myocytes were similar in normal and failing hearts. We were not able to patch spontaneously contracting human ventricular myocytes. Therefore, we did not measure any spontaneously contracting myocytes, although we were investigating the pacemaker current. A total of 133 cells yielded results for these experiments; mean cell capacity was 223.7±7.3 pF.

**Solutions**

Cells were superfused with a “standard” Tyrode’s solution containing (mmol/L) CaCl₂ 2.0, NaCl 115, KCl 25, MgCl₂ 1, BaCl₂ 8 (unless indicated), CdCl₂ 0.3, 4-aminopyridine 3, HEPES-NaOH 10; pH was adjusted to 7.3 with NaOH, 37°C), followed by 40 minutes with the same solution with added collagenase (type II, 200 IU/mL; Worthington) and protease (type XIV, 0.3 IU/mL; Sigma Chemical Co). Finally, the enzyme was washed out for 15 minutes with modified Tyrode’s solution that contained 100 μmol/L CaCl₂. Cells used in this study were taken from the central part of the myocardial wall. Cells were disaggregated by mechanical agitation and, after filtering through a nylon mesh, were stored at room temperature in Tyrode’s solution containing 2.0 mmol/L CaCl₂.

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**Recording Techniques**

Experiments were carried out by use of standard microelectrode whole-cell patch-clamp technique using an axopatch 200-B amplifier (Axon instruments). Microelectrodes were pulled from borosilicate glass and had tip resistances of 2 to 4 MΩ when filled with the pipette solution. All voltage recordings were corrected for the liquid junction potentials (range, −4.9 mV for the solution containing [K⁺], 5 mmol/L to −1.1 mV for the solution containing [K⁺], 140 mmol/L). A pipette with multiple superfusion lines was positioned over the cell studied to allow fast solution changes. Experiments were performed at a temperature of 22.0±0.5°C (unless indicated).

Analog filtering of current recordings was done at 3 kHz. Currents were digitized and stored for off-line analysis (pclamp 6.0, Axon instruments). Cell capacitance was calculated in each cell by applying hyperpolarizing 10-mV steps from a holding potential of −80 mV and integrating the current required to charge the membrane when stepping back to −80 mV.

**Statistical Analysis**

If size was measured as the difference between the instantaneous current at the beginning of the hyperpolarizing step and the steady-state current at the end of hyperpolarization. Currents were normalized to membrane capacitance to calculate current densities when indicated. Specific conductance of If was determined for each cell according to the equation g = I/[(Vh − Vrest)], where g is the conductance calculated at the membrane potential Vh, If is the current amplitude, and Vrest is calculated from the analysis of tail currents. For calculation of steady-state activation curves, specific current conductances were normalized to the maximal current conductance to give g/gmax. Boltzmann distributions were fitted to these normalized values: g/gmax = 1/[1 + exp{(Vm − V1/2)/S}], where Vmax is the membrane voltage, V1/2 is the voltage at half-maximal activation, and S is a slope factor at Vmax = V1/2. The relative permeabilities of potassium and sodium (PNa/K) were estimated by fitting the Goldman-Hodgkin-Katz equation23 to the reversal potential (Vrev): Vrev = −58.3 log{(PNa × [Na⁺])/(PK × [K⁺])}. Dose dependence of the adenosine effect was calculated by fitting a Hill function to the average shift at half-maximal activation (γ): Y = Ymax/(1 + Kᵢ/[Ado]), where Kᵢ is the adenosine concentration giving a half-maximal effect, [Ado] is the adenosine concentration, and H is the Hill coefficient. Data are presented as mean±SEM when appropriate. The Mann-Whitney nonparametric test was used for statistical evaluation, and values of P<0.05 were considered significant.

**Results**

**Characteristics of If**

Initially, we investigated the basal characteristics of If in human ventricular myocytes. Fig 1A shows a typical original current recording of If in a single human ventricular myocyte in “standard” Tyrode’s solution containing [K⁺], 25 mmol/L. From a holding potential of −40 mV, a family of hyperpolarization steps in 10-mV increments elicited a time-dependent inward current that increased with more negative potentials. If could be recorded in all cells investigated (n=133). Mean current densities in myopathic cells at −80 mV and −130 mV were −0.15±0.01 pA/pF and −1.38±0.14 pA/pF, respectively (n=59). A Boltzmann distribution, which was fitted to normalized current conductances of these 59 cells, showed current activation first at approximately −80 mV (Fig 1B). Half-maximal activation and slope factor were −110.96±0.06 mV and −12.26±0.06 mV⁻¹, respectively (n=59). To evaluate, whether there was any significant temperature dependence over a range of 22°C to 37°C, we also investigated 21 myopathic myocytes under similar conditions at a temperature of 37±0.5°C. At 37±0.5°C, current densities at −80 and −130 mV were not significantly different from results obtained.
at 22°C. However, consistent with observations in the rabbit sinoatrial node and in sheep Purkinje fibers, current activation was faster at higher temperatures with a Q10 of 2.28 ± 0.13.

Similar to mammalian pacemaker cells and ventricular myocytes, the current amplitude increased with increasing external K⁺ concentrations. In [K⁺]o, 5 mmol/L, current densities at −80 and −130 mV were −0.03 ± 0.01 and −0.47 ± 0.06 pA/pF (n=39); in [K⁺]o, 140 mmol/L, they were −0.64 ± 0.15 and −3.95 ± 0.57 pA/pF (n=16), respectively. Extracellular addition of Cs⁺ 2 mmol/L suppressed the time-dependent part of the inward current (n=4). Fig 2 depicts original current traces of a single myocyte in “standard” Tyrode’s solution before (Fig 2A) and during (Fig 2B) the addition of external Cs⁺. Consistent with results reported for other mammalian cardiac tissue, extracellular Cs⁺ did not affect outward tail currents. On removal of [Cs⁺]o, the Cs⁺-dependent block was partially reversible.

Tail current recordings were used to evaluate the reversal potential (Vrev) of Iᵢ. Tail currents, after a hyperpolarizing step to −120 mV, were elicited by 10-mV steps to 40 to −40 mV. Normalized tail current amplitudes from 13 cells in 25 mmol/L [K⁺]o are plotted as a function of tail step potential in Fig 3. Data points were fitted by a single linear function with a reversal potential of −16.80 ± 0.32 mV (slope factor, 0.021 mV⁻¹; r²=.97 for goodness of fit). The relative permeabilities of potassium and sodium (PNa/K) were estimated by fitting the Goldman-Hodgkin-Katz equation to Vrev. PNa/K calculated for a [Na⁺], range of 1 to 10 mmol/L was 0.41 to 0.43. Because results in rabbit sinoatrial node cells and canine ventricular myocytes indicated that Iᵢ might exhibit outward rectifica-

Figure 1. Voltage and time dependence of Iᵢ. A, Original current recordings show the voltage and time dependence of the hyperpolarization-activated inward current in a single human ventricular myocyte (myopathic). Hyperpolarization steps were applied from a holding potential of −40 mV to −70 to 160 mV. External solution contained (mmol/L) KCl 25, NaCl 115, BaCl₂ 8, CdCl₂ 0.3, and 4-aminopyridine 3. B, Activation curve was calculated by fitting a Boltzmann distribution to normalized current conductances of 59 cells at 22±0.5°C. First current activation occurred at approximately −80 mV; half-maximal activation was −110.96 ± 0.06 mV. Points represent mean±SEM.
tion, we also fitted the inward (slope, 0.013 mV$^{-1}$; $r^2=.99$) and outward (slope, 0.027 mV$^{-1}$; $r^2=.98$) sections with two separate linear functions, yielding a $V_{rev}$ of $-12.27 \pm 0.30$ mV and a $P_{Na/K}$ ratio of 0.53 to 0.56. Because the goodness of fit was not significantly different, we used the simplified single linear approximation for further evaluations.

**Effect of $\beta$-Adrenergic Stimulation**

To investigate the effect of $\beta$-adrenergic stimulation, 36 cells (31 myopathic, 5 nonfailing) were hyperpolarized in "standard" Tyrode’s solution before and after the addition of $10^{-5}$ mol/L isoproterenol. Isoproterenol shifted current activation to more positive potentials without changing the maximal current amplitude and accelerated current activation in myopathic (Fig 4A) and nonfailing cells. At potentials more positive to $-110$ mV, current activation followed a single-exponential function. Time constants at $-100$ mV before and after the addition of isoproterenol were $744.7 \pm 17.4$ and $703.1 \pm 13.5$ months, respectively ($n=36$) ($P=NS$). At more negative voltage steps, $I_f$ exhibited a sigmoidal time course with an initial delay in activation and was best fitted by a double-exponential function (at $-130$ mV: $\tau_{fast}$ $98.5 \pm 3.5$ and $72.4 \pm 2.6$ months, $P<.05$; $\tau_{slow}$, $810 \pm 28$ and $824 \pm 29$ months in the absence and presence of isoproterenol, respectively; $n=36$). Activation curves before and during isoproterenol application were obtained with hyperpolarizing steps to potentials between $-60$ and $-160$ mV (Fig 4B). Activation parameters, calculated by Boltzmann fits of normalized current conductances, showed that isoproterenol $10^{-5}$ mol/L shifted the potential of half-maximal activation by $10.30 \pm 0.28$ mV (from $-110.42 \pm 0.23$ mV to $-100.12 \pm 0.30$ mV; $n=36$; $P<.05$) without a significant difference between myopathic ($10.17 \pm 0.32$ mV; $n=31$) and nonfailing ($10.40 \pm 0.48$ mV; $n=5$) cells. There was no difference in the current reversal potential in the absence ($-16.1 \pm 0.9$ mV) and presence ($-15.2 \pm 0.6$ mV) of isoproterenol ($n=4$; $P=NS$).

**Modulation of $I_f$ by Carbachol and Adenosine**

In rabbit sinus node cells, $I_f$ was found to be directly modulated by acetylcholine and adenosine via muscarinic and A1-recep-
tors, respectively. Therefore, we investigated the effects of carbachol and adenosine on If in human ventricular myocytes under basal conditions. Cells were first hyperpolarized in standard Tyrode’s solution and then in the same solution after the addition of carbachol 10^4 mol/L or adenosine 10^5 mol/L and after washout. No direct effect of carbachol (n=9; 6 myopathic, 3 controls) or adenosine (n=15; 12 myopathic, 3 controls) was observed.

Because If in human ventricular myocytes was found to be stimulated by isoproterenol, we evaluated the indirect effects of carbachol and adenosine on prestimulated If. Thus, after hyperpolarization in standard Tyrode’s solution, cells were exposed to standard Tyrode’s solution containing isoproterenol 10^5 mol/L. Then the myocytes were hyperpolarized in the same isoproterenol solution in the presence of carbachol 10^4 mol/L or adenosine 10^5 mol/L and after washout. Both carbachol and adenosine reversibly antagonized the stimulating β-adrenergic shift of current activation. Steady-state activation curves were calculated by Boltzmann distributions, which were fitted to normalized current conductances. Carbachol (10^−3 mol/L) shifted the potential of half-maximal activation by −8.44±0.38 mV from −101.52±0.72 to −109.95±0.34 mV (n=7; 5 myopathic, 2 controls; P<.05) (not shown). Half-maximal activation before isoproterenol stimulation (−110.20±0.48 mV) and during carbachol exposure was not significantly different. There was no change in response to carbachol in myopathic and control cells. The current reversal potential was unchanged before (−15.8±0.4 mV) and during (−15.3±0.8 mV) carbachol application (n=3; P=NS).

After isoproterenol prestimulation, adenosine was also found to reversibly shift current activation to more negative potentials (Fig 5A). From Fig 5A, it is also evident that adenosine antagonized acceleration of current activation by isoproterenol. Fig 5B shows activation curves obtained by Boltzmann fits of 9 myocytes (7 myopathic, 2 controls) in standard Tyrode’s solution without and with added isoproterenol (10^−5 mol/L) alone and with added isoproterenol plus adenosine (10^−5 mol/L). Adenosine shifted the (prestimulated) potential of half-maximal activation from −100.74±0.59 to −107.22±0.33 mV (n=9; P<.05). Thus, adenosine did not completely reverse the stimulating effect of isoproterenol, although the difference in half-maximal activation in standard Tyrode’s solution (−109.13±0.43 mV) and in the presence of adenosine was not significantly different. No difference in response to adenosine was obtained between myopathic and nonfailing cells. The reversal potential was similar before (−15.5±0.6 mV) and during (−15.2±0.4 mV) exposure to adenosine (n=5; P=NS).

The dose dependence of the adenosine effects was examined by exposing cells to adenosine concentrations of 0.1 (n=3), 0.3 (n=4), 1 (n=3), 3 (n=7), 10 (n=9), 30 (n=5) and 100 (n=2) μmol/L. The adenosine effect was measured as the shift of the activation curve. Fig 6 depicts a Hill function, which was fitted to the average shift values. The Hill function yielded a K_h of the adenosine effect of 2.12±0.33 μmol/L, a maximal shift of −6.75±0.29 mV, and a Hill coefficient (h) of 1.76.

**If in Patients With Heart Failure Versus Undiseased Controls**

To evaluate whether there was any overexpression of If in human heart failure, normalized current densities (at −130 mV) measured in cells from patients with heart failure were compared with cells from donor hearts. Average current densities in myocytes from patients with dilated cardiomyopathy (−1.37±0.12 pA/pF; n=50) and ischemic cardiomyopathy (−1.39±0.24 pA/pF; n=30) were larger than in nonfailing controls (−1.18±0.21 pA/pF; n=24). This observation was also true when current densities were compared at 22°C and 37°C separately. However, because of the variation in current densities, these differences did not reach statistical significance (P=.23). We did not observe any difference of activation threshold between myopathic cells and nonfailing controls.

Because the inward rectifier current (I_k) contributes significantly to the stabilization of the resting membrane potential, we investigated myocytes in physiological potassium concentration ([K^+]_o, 5 mmol/L) in the absence and in the presence of external Ba^2+. Fig 7 depicts original current recordings of a single ventricular myocyte, demonstrating the magnitude of the outward current I_k, at potentials positive to −80 mV in relation to the inward current (I). Mean current densities of the outward current (I_k) obtained in 13 myopathic cells at −70 and −60 mV were 0.02±0.07 and 0.29±0.07 pA/pF, respectively. Mean current density of If at −80 mV in the same cells was −0.03±0.01 pA/pF (n=13).

**Discussion**

This report describes the presence of a hyperpolarization–activated inward current in human ventricular myocytes. The current typically activates at potentials negative to −80 mV, is time and voltage dependent, and can be suppressed by the addition of extracellular cesium. Reversal potentials are consistent with a permeability for the monovalent cations Na^+ and K^+. The relative permeability P_{Na}/P_{K} was in the same range as in canine ventricular myocytes at comparable ionic concentrations. Thus, this current has properties similar to the pacemaker current I_k, with I_k has been observed in sinus node,
frog sinus venosus, AV node, atrium, Purkinje fibers, and mammalian ventricular myocytes of the guinea pig, dog, and rat. In pacemaker cells, If is believed to be the major current determining the diastolic depolarization phase. In sinoatrial node cells, If activates at more positive potentials than in Purkinje cells. But in both tissues, activation occurs within the physiological diastolic voltage range. In mammalian ventricular myocytes, results are controversial. In guinea pig and canine ventricular cells, the activation threshold for If ranged between $-105$ and $-140 \text{ mV}$, much more negative than the potassium equilibrium potential ($E_K$). In rat ventricular myocytes, however, If first activated at voltages ($-60 \text{ mV}$) overlapping the resting membrane potential range. In hypertrophic ventricular myocardium of the rat, If density was significantly higher than in control animals. The authors postulated that overexpression of If in hypertrophied rat myocardium might be an important arrhythmogenic mechanism in these animals.

Because electrophysiological alterations like prolongation of the action potential and reduction in $I_o$ were found to be similar in animal models of heart failure and in human heart failure, we especially wanted to determine whether patients with heart failure might also have an overexpression of If. The existence of If in human ventricular myocytes has previously been reported only in a limited number of patients in abstract form by our group and as a Brief Rapid Communication by Cerbai et al. However, data concerning the potential pathophysiological role of If in the failing human heart compared with undiseased myocardium are still lacking. Therefore, we recorded If in isolated myocytes of patients with

**Figure 4.** Effect of $\beta$-adrenergic stimulation. A, Superimposed original current recordings of a single ventricular myocyte (myopathic) in “standard” Tyrode’s solution (control) and in the same solution to which isoproterenol $10^{-5} \text{ mol/L}$ (ISO) had been added. Isoproterenol led to a shift in current activation to more positive potentials without a change in maximal current amplitude and accelerated current activation. B, Activation curves calculated by Boltzmann fits of normalized current conductances showed that isoproterenol $10^{-5} \text{ mol/L}$ shifted the potential of half-maximal activation by $10.30 \pm 0.28 \text{ mV}$ (from $-110.42 \pm 0.23$ to $-100.12 \pm 0.30 \text{ mV}$; $n=36$; $P<.05$).
terminal heart failure caused by dilated or ischemic cardiomyopathy and compared these results with nonfailing controls. Our preliminary data obtained in a small number of patients suggested a larger current in the failing human heart. The present results obtained in a much larger group of patients supported our previous findings. Cells isolated from hearts of patients with terminal heart failure were found to have larger average current densities than myocytes from undisease donor hearts. However, this difference did not reach statistical significance because of large current variations. We did not find any difference in activation threshold between myopathic and undisease myocytes. First current activation and half-maximal activation were observed at approximately $280$ and $2110$ mV, respectively. Thus, similar to rats, activation of I_f in human ventricular myocytes occurs at voltages near the diastolic membrane potential, and there seems to be at least a tendency toward an increased current size in the failing human heart.

However, to estimate the potential functional relevance of I_f in human heart failure, autonomic regulation has to be considered. In various mammalian cardiac tissue, I_f was found to be increased by β-adrenergic stimulation and decreased by muscarinic agonists via a shift in current activation to more positive or negative potentials, respectively. In sinus node preparations, β-adrenoceptor agonists increased the slope of phase 4 diastolic depolarization and enhanced automaticity, whereas muscarinic stimulation slowed the pacing rate. In rat ventricular myocytes, isoproterenol $10^{-5}$ mol/L shifted activation of I_f by approximately 10 mV. In dog ventricular cells, the phosphatase inhibitor calyculin A led to a maximal current shift of 30 mV. In human ventricular myocytes, we found a shift in current activation by 10 mV after β-adrenergic stimulation with isoproterenol $10^{-3}$ mol/L. Unlike in rabbit sinus node cells and sheep and rabbit Purkinje cells, the muscarinic agonist carbachol was found to have no direct effect on I_f in human ventricular myocardium. However, similar to canine Purkinje fibers, carbachol antagonized the stimulating action of isoproterenol. Thus, muscarinic agonists seem to have a negative feedback function that might protect the cells from I_f increase caused by β-adrenergic stimulation. However, patients with heart failure are known to have an increased

Figure 5. Effects of adenosine ($10^{-5}$ mol/L) on β-adrenergic prestimulated I_f. A, Superimposed current traces of a single myocyte (myopathic cell) recorded in “standard” Tyrode’s solution containing isoproterenol $10^{-5}$ mol/L (ISO) and in the same isoproterenol solution with added adenosine $10^{-5}$ mol/L (ADO+ISO). Adenosine led to a shift in current activation to more negative potentials and slowed activation kinetics but had no effect on maximal current size. B, Normalized current amplitudes under basal conditions in the presence of isoproterenol ($10^{-5}$ mol/L) alone and in the presence of both isoproterenol and adenosine ($10^{-5}$ mol/L) were fitted by Boltzmann distributions ($n=9$). Adenosine led to a shift in half-maximal current activation from $-100.74 \pm 0.59$ to $-107.22 \pm 0.33$ mV ($P<.05$). Points represent mean ± SEM.
sympathetic and reduced parasympathetic tone. In patients with heart failure, plasma norepinephrine levels are increased. Additionally, a depressed heart rate variability, an indirect measure of higher sympathetic tone, has been observed in these patients. In our experiments, β-agonists stimulated If in myopathic cells despite the known β-receptor downregulation in the failing human heart. Therefore, the higher sympathetic tone in patients with heart failure is likely to be of special importance, because it might lead to a shift in If activation to more positive potentials in vivo and thus to a further current increase.

In addition to the autonomic β-sympathomimetic and muscarinic systems, the endogenous nucleoside adenosine plays a physiological role in the modulation of cardiac function. In mammalian and human myocardium, adenosine binds to specific A1-receptors. Depending on the species and the type of myocytes, A1-receptors are coupled to various ionic channels via G proteins in a direct way or an indirect, cAMP-dependent way, antagonizing the effects of catecholamines. In rabbit sinoatrial myocytes, Zaza et al. found a direct effect of adenosine on basal I1 and a direct reduction in the pacing rate. We did not observe any direct modulation of If by adenosine in human ventricular myocytes. However, similar to the mode of I1 inhibition by carbachol, adenosine attenuated the stimulating effect of β-agonists in human ventricular myocardium. This mode of adenosine action is consistent with the negative inotropic effect of adenosine in human ventricular myocardium described by Böhm et al. These authors also observed only an indirect negative inotropic effect but no direct action of adenosine. Under physiological conditions, atrial and ventricular myocardial cells release adenosine at concentrations of 0.1 to 1 μmol/L. Therefore, adenosine concentrations that showed inhibitory effects on If in our experiments were in the physiological range. Under pathological conditions, such as ischemia, increased cardiac workload, or heart failure, elevated adenosine concentrations have been measured. However, in our experiments, adenosine did not entirely antagonize the β-adrenergic stimulating effect on If even at high adenosine concentrations (shift by isoproterenol, 10 mV; maximal shift by adenosine, 2 mV), although this difference was not statistically significant. Cerbai et al. observed first activation of If in 24 ventricular myocytes of patients with dilated cardiomyopathy at approximately −55 mV. This difference in current threshold com-
pared with our results may partially be due to the liquid junction potential for which their data apparently were not corrected (approximately −16 mV). Additionally, the higher Ca^{2+} concentration in the pipette solution that these authors used may have contributed to this difference in I_{f} threshold. Elevation of intracellular Ca^{2+} from Pca 10 to 7 shifted the I_{f} activation curve by 13 mV in rat sinoatrial node cells, although not by a direct effect on I_{f} channel.\(^{68,69}\) In our experiments, intracellular Ca^{2+} was buffered and Ca^{2+} transients were abolished (EGTA in the pipette solution, extracellular Ca^{2+} to block Ca^{2+} currents, Na^{+}-free pipette solution to block Ca^{2+} influx through the Na^{+}/Ca^{2+} exchange system) to investigate pure I_{f} and to avoid any possible influence of altered intracellular Ca^{2+} handling on current measurements. However, in patients with end-stage heart failure, an increased diastolic [Ca^{2+}], has been described.\(^{17,18,70}\) This alteration in diastolic Ca^{2+} may further increase I_{f} in vivo in addition to the effects of elevated sympathetic tone.

We were not able to patch any spontaneously contracting cells. Because I_{f} is known to contribute to diastolic depolarizations in pacemaker cells, I_{f} might have been larger in these spontaneously contracting myocytes. This might have led to an underestimation of current size in our experiments. However, by face value, there was no difference in the number of spontaneously beating cells between failing and control hearts. In addition, the frequency of oscillations was slow, and some myocytes showed “waveform” contractions. Thus, we suppose that in a significant proportion of these cells, spontaneous contractions were caused by calcium overload in a significant proportion of these cells, spontaneous contractions were caused by calcium overload after the isolation procedure rather than by the pacemaker current If.\(^{11}\) Contractile oscillations in pacemaker cells, I_{f} might have been larger in these conditions I_{f} and Ca^{2+} currents. Divalent cations are known to shift the activation curves of most voltage-dependent channels on the surface membrane,\(^{71–74}\) including I_{f} by different amounts in the depolarizing direction through surface charge screening and/or binding. Because low barium concentrations (2 mmol/L) do not block I_{K1} effectively in human ventricular myocytes, it is difficult to measure the shift in I_{f} activation caused by [Ba^{2+}], in these cells. However, it is known from the literature that in sinoatrial node preparations\(^{75,76}\) and Purkinje fibers,\(^{78–80}\) elevation of [Ca^{2+}], from physiological calcium concentrations by 5.4 to 10.0 mmol/L led to a shift in I_{f} activation by 4 to 10 mV and that barium exhibits fewer effects on the surface potential than calcium.\(^{72,74,81}\) Estimated from these data, [Ba^{2+}], 8 mmol/L and [Cd^{2+}], 0.3 mmol/L in our external solutions to suppress the interference of I_{K1} and Ca^{2+} currents. Divalent cations are known to shift the activation curves of most voltage-dependent channels on the surface membrane,\(^{71–74}\) including I_{f} by different amounts in the depolarizing direction through surface charge screening and/or binding. Because low barium concentrations (2 mmol/L) do not block I_{K1} effectively in human ventricular myocytes, it is difficult to measure the shift in I_{f} activation caused by [Ba^{2+}], in these cells. However, it is known from the literature that in sinoatrial node preparations\(^{75,76}\) and Purkinje fibers,\(^{78–80}\) elevation of [Ca^{2+}], from physiological calcium concentrations by 5.4 to 10.0 mmol/L led to a shift in I_{f} activation by 4 to 10 mV and that barium exhibits fewer effects on the surface potential than calcium.\(^{72,74,81}\) Estimated from these data, [Ba^{2+}], 8 mmol/L and [Cd^{2+}], 0.3 mmol/L would be expected to shift I_{f} activation by ~8 mV under our experimental conditions. However, this value has to be compensated for further for the barium-induced dose- and voltage-dependent block of I_{f}.\(^{82}\) In sinoatrial node cells, external barium (3 to 5 mmol/L) decreased I_{f} amplitude and shifted the midactivation potential to more negative voltages.\(^{82–83}\) More than half of I_{f} activation shift caused by [Mn^{2+}], was compensated for the addition of [Ba^{2+}], (1 mmol/L).\(^{76}\) Thus, the use of divalent cations in our experiments (predominantly [Ba^{2+}], in addition to physiological concentrations of [Ca^{2+}], and [Mg^{2+}],) is unlikely to have caused a significant overestimation of I_{f} amplitude but might rather have led to an underestimation of I_{f} size, especially at low negative potentials.

In discussions of the functional significance of I_{f} in human myocardium, I_{f} also has to be compared with other currents, especially the inward rectifier current (I_{K1}). Negative to the potassium equilibrium potential (E_{K}), the stabilizing effect of I_{K1} will drive the membrane potential back to normal resting values. However, at voltages positive to E_{K}, the outward current density of I_{K1} is rather small. Additionally, the density of I_{K1} is reduced by ~40% in cells from myopathic ventricles compared with undiseased controls.\(^{16,84}\) Although in most myocytes I_{K1} seems to be larger than I_{f} at physiological potentials, the combination of reduced I_{K1} size and increased I_{f} size in the failing human heart might favor diastolic depolarizations in individual cells.

In conclusion, patients with end-stage heart failure have a trend toward increased I_{f} densities compared with nonfailing control hearts. Additionally, the elevated sympathetic tone and increased diastolic [Ca^{2+}], might further increase I_{f} in these patients. Together with a reduced current density of I_{K1} in heart failure, I_{f} might drive the membrane potential toward threshold in individual cells in the failing human heart. However, further studies are necessary to test whether under these conditions I_{f} can lead to spontaneous diastolic depolarizations in vivo.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (Be 1113/2–3) and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (01 KS 9502, ZMMK Projekt 4). We thank I. Beckmann for assistance with cell isolation and I. Priebe for comments on the manuscript.

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Hyperpolarization-Activated Inward Current in Ventricular Myocytes From Normal and Failing Human Hearts

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Circulation. 1998;97:55-65
doi: 10.1161/01.CIR.97.1.55

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
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