Single-Channel Properties Support a Potential Contribution of Hyperpolarization-Activated Cyclic Nucleotide-Gated Channels and $I_f$ to Cardiac Arrhythmias

Guido Michels, MD; Fikret Er, MD; Ismail Khan, PhD; Michael Südkamp, MD; Stefan Herzig, MD; Uta C. Hoppe, MD

Background—The pacemaker current $I_f$ is present in atrial and ventricular myocytes. However, it remains controversial whether $I_f$ overexpression in diseased states might play a role for arrhythmogenesis, because first $I_f$ activation in whole-cell recordings hardly overlapped the diastolic voltage of working myocardium.

Methods and Results—To obtain further insight into $I_{HCN}$ and $I_f$ properties, we provide for the first time detailed single-channel analysis of heterologously expressed hyperpolarization-activated cyclic nucleotide-gated (HCN) isoforms and native human $I_f$. HCN subtypes differed significantly in single-channel amplitude, conductance, and activation kinetics. Interestingly, threshold potentials of HCN isoforms were more positive than would have been expected from whole-cell measurements. Single-channel properties of cells cotransfected with HCN2 and HCN4 were distinct from cells expressing HCN2 or HCN4 alone, demonstrating that different HCN isoforms can influence current properties of a single HCN channel complex, thus providing direct functional evidence for HCN heteromerization. Pooled data of homomeric and heteromeric HCN channels and of native $I_f$ extrapolated from maximum likelihood fits indicated a multistate gating scheme comprising 5 closed- and 4 open-channel states. Single-channel characteristics of $I_f$ in human atrial myocytes closely resembled those of HCN4 or HCN2+HCN4, supporting the hypothesis that native $I_f$ channels in atrial myocardium are heteromeric complexes composed of HCN4 and/or HCN2. Most interestingly, half-maximal activation of single-channel atrial $I_f$ ($-68.3\pm4.9$ mV; $k=-9.9\pm1.5$; $n=8$) was well within the diastolic voltage range of human atrial myocardium.

Conclusions—These observations support a potential contribution of HCN1/$I_f$ to the arrhythmogenesis of working myocardium under pathological conditions. (Circulation. 2005;111:399-404.)

Key Words: pacemakers  ■  electrophysiology  ■  ion channels  ■  arrhythmia  ■  myocytes

The pacemaker current $I_f$ is present in both cardiac automatic and nonautomatic tissue. In sinoatrial node cells and neonatal cardiacocytes, $I_f$ contributes significantly to spontaneous diastolic depolarization. In heart failure, hypertrophy, and atrial fibrillation, $I_f$ current densities and/or mRNA levels of its molecular correlate hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are increased compared with controls. However, it remains controversial whether $I_f$ overexpression might play a role for the increased propensity of arrhythmias in diseased states because $I_f$ current activation was obtained at more negative potentials in working myocardium than in pacemaker cells. Four HCN gene family members have been cloned, 3 of which are present in heart (HCN1, HCN2, HCN4) with varying message levels in different cardiac regions. By functional interference, with the use of the yeast 2-hybrid system, and by dominant-negative constructs, it has been proposed that HCN isoforms can coassemble to form heteromeric channel complexes like voltage-gated K+ channels. However, direct functional evidence of HCN heteromultimerization is still lacking.

Precise understanding of the molecular structure and function of the pacemaker channel is critical to any future therapeutic modulation of this current in myocardium. Therefore, to obtain further insight into $I_{HCN}$ and $I_f$ properties, we provide for the first time detailed single-channel analysis of heterologously expressed HCN isoforms and native human $I_f$. Most surprisingly, activation of single HCN1/$I_f$ channels was observed at more positive potentials and single-channel amplitudes were larger than previously reported during whole-cell driven activation. These observations support a potential role of HCN1/$I_f$ for the arrhythmogenesis of working myocardium under pathological conditions.
Methods

Plasmid Construction and Transient Transfections

The expression plasmids pAdCGI-HCN1, pAdCGI-HCN2, and pAdCGI-HCN4 encoding the full-length sequences of mHCN1, mHCN2, and hHCN4, respectively, and pAdCGI have been described. Twenty-four hours before transfection, CHO-K1 cells (ATCC CCL 61, American Type Culture Collection, Manassas, Va) were seeded at a density of 2.0×10^4 cells/mL. CHO-K1 cells were transfected with 0.5 μg/well plasmid DNA (as indicated) with the use of Lipofectamine Plus (Life Technologies, Gaithersburg, Md) as directed by the manufacturer. After 4 hours, transfection media were replaced with normal growth media.

Myocyte Isolation

Seventeen right atrial appendage specimens were obtained from patients (12 men, aged 64.0 ± 2.6 years; 5 women, aged 67.4 ± 4.5 years) undergoing coronary bypass surgery. A standard isolation method was used to prepare atrial myocytes as previously described. The local ethics committee approved experimental use of tissue samples.

Electrophysiology

Single-channel experiments were performed with the use of standard microelectrode cell-attached patch-clamp techniques with an Axopatch 200B amplifier and Digidata 1200 interface (Axon Instruments) at room temperature (21°C to 23°C) during sampling at 10 kHz and filtering at 2 kHz (−3 dB, 4-pole Bessel). After seal formation, capacitive currents of the membrane (range, 15 to 105 pF) were compensated to a minimum with the use of the capacitance neutralization circuit implemented in the Axopatch 200B amplifier. Single HCN/II channels were recorded at 2000-fold amplification. The bath solution contained the following (mmol/L): KCl 130, NaCl 10, EGTA 5, HEPES-KOH 10 (pH 7.4 with KOH). This high-K solution was used to achieve a resting membrane potential of zero. Pipettes (7 to 10 MΩ) were filled with the following (mmol/L): KCl 70, NaCl 70, MgCl2 1, BaCl2 2, HEPES-KOH 5 (pH 7.4 with KOH). For Iod recordings of cardiomyocytes, CdCl2 200 μmol/L and 4-amiporphidrine 4 mmol/L were added to block the L-type calcium channel (I_{ca, L}) and transient outward current (I_{to}), respectively. In some experiments forskolin (50 μmol/L; Sigma) was added to the bath solution. Single channels were depolarized or hyperpolarized (as indicated) at continuous pulse mode for a total duration of 3 seconds (20×150 ms sweeps) from a holding potential of −35 mV. Data were corrected for the liquid junction potential of −2.1 mV. A xenon arc lamp was used to view EGFP at 488/530 nm (excitation/emission).

Data Analysis

Single-channel measurements and analysis were done with the use of custom software as previously reported. Linear leak and capacity currents were digitally subtracted with the average currents of nonactive sweeps. For detailed gating analysis, idealized currents were analyzed in 150-ms steps recorded at continuous pulse mode (total recording 20×150 ms). Closed-time and first-latency analyses were performed only in 1-channel patches. The open probability (defined as the relative occupancy of the open state during active sweeps), availability (fraction of sweeps containing at least 1 channel opening), and I_{ca, L} (as the peak ensemble average current, obtained using Boltzmann fits yielded half-maximal activation voltages (V_{0.5}) of −51.2 ± 6.8 mV for HCN1 (k=−13.0±1.0, n=5), −58.5 ± 4.8 mV for HCN2 (k=−15.8±3.0, n=6), and −74.2±5.2 mV for HCN4 (k=−12.3±1.6, n=7) (Figure 1B). Surprisingly, threshold potentials of HCN isoforms were observed at more positive values than would have been expected from whole-cell measurements from our laboratory and others. Indeed, first activation of HCN1 and HCN2 was recorded as positive at −30 mV in individual cells (range, HCN1 −30 to −50 mV, n=6; HCN2 −30 to −50 mV, n=13; HCN4 −50 to −70 mV, n=10), thus being well in the diastolic voltage range of native pacemaker cells and working myocardium. Consistent with whole-cell measure-

Results

Distinct Single-Channel Properties of HCN Isoforms

To characterize single-channel properties of cardiac HCN isoforms, CHO cells were transfected with expression plasmids encoding the full-length sequences of HCN1, HCN2, or HCN4. No hyperpolarization-activated current was observed in control cells transfected with empty expression vectors. Original current recordings (Figure 1A) and mean data (Table) clearly demonstrate that HCN isoforms differed significantly in single-channel amplitudes: HCN2>HCN1>HCN4. Consistent with amplitude measurements, slope conductance calculated by linear regression of individual recordings varied profoundly among HCN subtypes, with HCN2 exhibiting the highest conductance (Table). Interestingly, single-channel conductance of all HCN isoforms was markedly larger than previously reported for native I_{t} of sinoatrial node cells during whole cell–driven activation. In contrast to the whole-cell patch-clamp mode, no current rundown was observed in our cell-attached single-channel recordings. The reversal potential of HCN channels was close to that expected at similar sodium and potassium concentrations for native I_{t} (HCN1 −22.9 ± 7.4 mV, n=7; HCN2 −23.3±5.2 mV, n=12; HCN4 −21.1±5.3 mV, n=10). Boltzmann fits yielded half-maximal activation voltages (V_{0.5}) of −51.2±6.8 mV for HCN1 (k=−13.0±1.0, n=5), −58.5±4.8 mV for HCN2 (k=−15.8±3.0, n=6), and −74.2±5.2 mV for HCN4 (k=−12.3±1.6, n=7) (Figure 1B). Surprisingly, threshold potentials of HCN isoforms were observed at more positive values than would have been expected from whole-cell measurements from our laboratory and others. Indeed, first activation of HCN1 and HCN2 was recorded as positive at −30 mV in individual cells (range, HCN1 −30 to −50 mV, n=6; HCN2 −30 to −50 mV, n=13; HCN4 −50 to −70 mV, n=10), thus being well in the diastolic voltage range of native pacemaker cells and working myocardium. Consistent with whole-cell measure-

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ments, HCN isoforms exhibited marked differences in activation kinetics, with HCN1 activating most quickly and HCN4 most slowly (Table). These results indicate that HCN subtypes differ profoundly in their single-channel characteristics.

**Direct Functional Evidence for HCN Heteromultimerization**

Thus far, only indirect evidence indicating HCN heteromerization has been provided. Therefore, to obtain direct functional evidence, single-channel currents of cells cotransfected with HCN2 and HCN4 were recorded. Indeed, single-channel properties of cotransfected cells were distinct from cells expressing HCN2 or HCN4 alone. Although open probability, availability, and half-maximal activation ($V_{0.5}$, $-50.1 \pm 6.6$ mV, $k = -11.3 \pm 2.8$, $n = 7$; $P = NS$ versus HCN2, $P = 0.014$ versus HCN4) of HCN2+HCN4 were similar to HCN2, current amplitude and conductance closely resembled those of HCN4 (Figure 1; Table). The threshold potential of HCN2+HCN4 varied from $-30$ to $-50$ mV in individual recordings. These observations clearly demonstrate that different HCN isoforms can influence current properties of a single HCN channel complex, thus providing direct functional evidence for HCN heteromultimerization. It still must be determined which channel regions and sequences exert dominant effects on specific current characteristics.

**Native $I_f$ Activates Well in the Diastolic Voltage Range of Human Atrial Myocytes**

Given that HCN2 and HCN4 are the dominant mRNA transcripts in atrial myocardium,10,12 single-channel recordings of native atrial $I_f$ should exhibit properties similar to our recordings of recombinant HCN2 and/or HCN4. Indeed, single-channel characteristics of $I_f$ in human atrial myocytes closely resembled those of HCN4 or HCN2+HCN4 (Figure 1, Table), supporting the hypothesis that native $I_f$ channels in

### Summary of Single-Channel Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCN1</th>
<th>HCN2</th>
<th>HCN4</th>
<th>HCN2+HCN4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open probability, %</td>
<td>34.40±10.20†</td>
<td>32.6±8.80†</td>
<td>8.40±3.30*$|$</td>
<td>34.20±9.01</td>
</tr>
<tr>
<td>Availability, %</td>
<td>74.64±14.18</td>
<td>78.47±6.10†</td>
<td>58.16±5.97*$|$</td>
<td>76.50±5.90</td>
</tr>
<tr>
<td>Mean open time, ms</td>
<td>1.12±0.16†</td>
<td>1.15±0.15†</td>
<td>0.51±0.07†</td>
<td>0.79±0.17</td>
</tr>
<tr>
<td>Mean closed time, ms</td>
<td>0.62±0.09††</td>
<td>2.94±0.45*</td>
<td>2.05±0.30*</td>
<td>2.85±0.50*</td>
</tr>
<tr>
<td>Mean first latency, ms</td>
<td>8.66±2.05±†‡</td>
<td>37.24±6.27*</td>
<td>41.83±4.22*</td>
<td>43.02±3.96*</td>
</tr>
<tr>
<td>Amplitude, pA</td>
<td>1.40±0.12†</td>
<td>2.16±0.15*$|$</td>
<td>0.95±0.06*$|$</td>
<td>1.31±0.11††</td>
</tr>
<tr>
<td>Conductance, pS</td>
<td>12.91±0.92†§</td>
<td>34.63±2.43*$|$</td>
<td>17.38±1.66†</td>
<td>21.14±2.43†</td>
</tr>
<tr>
<td>$I_{peak}$, fA</td>
<td>678±212†</td>
<td>809±170†</td>
<td>107±43*$|$</td>
<td>483±137†</td>
</tr>
</tbody>
</table>

| Availability (%)                 | -130 to -100 | -130 to -100 | -130 to -100 | -130 to -100 |
| Mean open time, ms              | 1.12±0.16   | 1.15±0.15   | 0.51±0.07   | 0.79±0.17   |
| Mean closed time, ms            | 0.62±0.09   | 2.94±0.45   | 2.05±0.30   | 2.85±0.50   |
| Mean first latency, ms          | 8.66±2.05   | 37.24±6.27  | 41.83±4.22  | 43.02±3.96  |
| Amplitude, pA                   | 1.40±0.12   | 2.16±0.15   | 0.95±0.06   | 1.31±0.11   |
| Conductance, pS                 | 12.91±0.92  | 34.63±2.43  | 17.38±1.66  | 21.14±2.43  |
| $I_{peak}$, fA                  | 678±212     | 809±170     | 107±43      | 483±137     |

Parameters are shown as mean±SEM values. For closed time and latency, only experiments containing just 1 detected open level were used for calculation. HCN1 has by far the shortest mean first latency, indicating that HCN1 activates most quickly. While mean first latency values are similar for HCN2 and HCN4, availability as well as open probability and mean open time is smaller for HCN4 than HCN2, indicating slower activation of HCN4 compared with HCN2. Measurements were performed at standard conditions (holding potential = -35 mV, test potential = -90 mV). One-way ANOVA was computed for all parameters with post hoc tests.

* $P<0.05$ vs HCN1.
† $P<0.05$ vs HCN2.
‡ $P<0.05$ vs HCN4.
§ $P<0.05$ vs HCN2+HCN4.
|| $P<0.05$ vs atrial $l_i$. 

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**Figure 1.** Comparison of single recombinant HCN and human atrial $I_f$ channels. A, Single-channel currents of HCN1. Middle, 20 consecutive single tracings of each channel. Single channels were depolarized at continuous pulse mode for a total duration of 3 seconds (20×150-ms sweeps), with a holding potential of -35 mV and a test potential of -90 mV. Bottom, ensemble average current of 1 consecutive sweep of 3-second pulse duration. Bars=50 ms, 2.5 pA (unitary current traces), or 1 second, 0.5 pA (ensemble average current). B, Voltage dependence of activation was analyzed with the Boltzmann function: $Y=Y_{max}/[1+e^{(Y_{0.5}-V)/k)}$, where $V_{0.5}$ is the voltage of half-maximal activation and $k$ is the slope factor. Channel availability was determined from experiments recorded as in A and plotted against the test potential. For data, see text.
atrial myocardium are heteromeric complexes composed of HCN4 and/or HCN2. Most interestingly, half-maximal activation of single-channel atrial $I_f$ ($V_{1/2}^{HCN4} = 68.3 \pm 4.9$ mV, $k = 9.9 \pm 1.5$, $n=8$) was also similar to HCN4. The addition of forskolin (50 μmol/L) shifted the half-maximal activation from $-72.86 \pm 3.7$ mV to $-55.87 \pm 1.69$ mV ($n=4$, $P=0.006$), without significantly affecting the slope factor $k$ ($8.62 \pm 2.7$ mV [control] and $11.4 \pm 1.43$ mV [forskolin]), demonstrating that sympathetic stimulation could modulate $I_f$ (Figure 2). This indicated that native $I_f$ activates well within the diastolic voltage range of human atrial myocardium, which has been controversial on the basis of whole-cell measurements in recent years.$^{8,26}$ Thus, $I_f$ indeed might contribute to arrhythmogenesis in working myocardium.

**Gating Kinetics of $I_{HCN}$ and $I_f$**
For years it has been known that kinetic features of native $I_f$ channel activation cannot be described satisfactorily by the use of the second-order Hodgkin-Huxley model.$^{4,27}$ Recently, an allosteric model of voltage gating of HCN channels has been hypothesized from whole-cell recordings as a combination of displacement of voltage sensors (1 for each of the 4 subunits) and a closed-to-open transition involving concerted rearrangements of all 4 subunits of the tetrameric channel.$^{28}$

In this assumption, 5 closed and 5 open states were proposed. However, direct evidence for this model is still missing. Therefore, we analyzed the rapid-gating process of $I_{HCN}$ and native $I_f$ by means of closed-time and open-time distributions at a test potential of $-90$ mV, at which single-channel events could still be resolved. Pooled data of homomeric and heteromeric HCN channels and of native $I_f$ extrapolated from maximum likelihood fits given by the best-fit method$^{23}$ indeed confirmed a multistate scheme comprising 5 closed- and 4 open-channel states (Figure 3): HCN1-$\tau_{open}$ values (ms): 0.12, 0.83, 2.39, 9.02 ($n=4$); $\tau_{closed}$ values (ms): 0.17, 0.80, 4.59, 6.02, 25.25 ms ($n=3$); HCN2-$\tau_{open}$ values (ms): 0.13, 0.86, 2.24, 5.93 ($n=8$); $\tau_{closed}$ values (ms): 0.15, 0.90, 3.36, 5.52, 20.95 (n=5); HCN4-$\tau_{open}$ values (ms): 0.13, 0.72, 1.66, 1.86 ($n=8$); $\tau_{closed}$ values (ms): 0.13, 0.82, 2.09, 6.69, 25.73 (n=5); HCN2+HCN4-$\tau_{open}$ values (ms): 0.13, 0.70, 1.53, 4.01 ($n=7$); $\tau_{closed}$ values (ms): 0.17, 1.05, 5.69, 7.70, 23.75 (n=4); atrial $I_f$-$\tau_{open}$ values (ms): 0.13, 0.81, 1.29, 2.11 (n=5); $\tau_{closed}$ values (ms): 0.15, 1.05, 2.44, 2.63, 25.92 (n=5). Thus, our results substantiate an allosteric gating model for both recombinant HCN and native $I_f$ channels.

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**Figure 2.** Effect of forskolin on single-channel voltage-dependence of $I_f$ in human atrial myocytes. The addition of forskolin (50 μmol/L) significantly shifted the half-maximal activation to more positive potentials. Channel availability and voltage dependence were determined as in Figure 1. For data, see text.

**Figure 3.** Open-time (A) and closed-time (B) histograms of recombinant HCN and human atrial $I_f$ channels. Curves were generated by maximum-likelihood fitting of pooled data. After visual inspection of the fitted curves on the histogram, the best fit was further estimated from a multiple comparison with the use of an F statistical test on the sum of squared errors of the functions and with consideration of the LLR.$^{23}$ The analysis revealed a significant multistate gating model of at least 4 open states and 5 closed states. For data, see text. SQR indicates square root.
Discussion

There has been much controversy about the functional contribution of \( I_f \) to cardiac arrhythmias in diseased states, mainly because \( I_f \) activates at more negative voltages in working myocardium than in pacemaker tissue.\(^ {4,5,8,25} \) In the present study we performed for the first time detailed single-channel analyses of recombinant HCN and native \( I_f \), which strongly indicate a potential role of these channels for arrhythmogenesis. Furthermore, our results provide direct functional evidence for the modulation of 1 individual channel complex by different HCN isoforms and give new insights into HCN/\( I_f \) channel gating.

HCN genes are the major molecular component of native \( I_f \).\(^ {1–3} \) In heterologous expression, we demonstrate that recombinant HCN isoforms differ profoundly in their single-channel properties. Although some of these characteristics might have been expected from whole-cell recordings, first HCN activation was observed at more positive potentials than previously described.\(^ {1,2,18} \) Most likely, this difference is caused by the well-known effect of \( I_{\text{HCN}} \) rundown immediately after break-in in whole-cell recordings,\(^ 8 \) whereas in the present experiments using the cell-attached mode we prevented cell dialysis. Distinct recording techniques are also sufficient to explain the higher single-channel conductance in our recordings (13 to 35 pS) compared with previous measurements of sinuso-node cells (1 pS).\(^ {17} \) In contrast to the present experiments, former single-channel openings were driven during whole-cell activation, amplitudes were obtained from multichannel patches, and capacitative transients were not compensated for, all of which can result in an underestimation of current size.

Heterologous coexpression of HCN2 and HCN4 gave rise to single channels incorporating characteristics of both isoforms in 1 individual channel complex. These results provide direct functional evidence that HCN2 and HCN4 can coassemble to form heteromeric complexes. Thus far, heteromultimerization has only been proposed in whole-cell recordings of tandem heterodimers and HCN coexpression, which, however, cannot precisely discriminate heteromerization from a simple addition of individual isoform properties.\(^ {13,14} \) More recently, interaction of HCN subunits has been suggested indirectly with the use of dominant-negative constructs and the 2-yeast hybrid system.\(^ {1,15,16} \) Our observations now substantiate evidence that different HCN isoforms can coassemble and may suffice to explain the regional diversity of pacemaker current in cardiac tissue.

Early investigation of the kinetics of native \( I_f \) has identified features that are not compatible with second-order Hodgkin-Huxley gating models. These include a sigmoidal activation time course that cannot be described by a fixed power of an exponential at all voltages, sigmoidal deactivation, and removal of activation delay by conditioning prehyperpolarizing steps.\(^ {4,29} \) Given the structural similarities of HCN and Kv channels, a multistate model accounting for a tetrameric channel complex whose closed/open transitions are regulated by voltage sensors should better describe HCN kinetics.\(^ {30} \) Consistent with these assumptions, multieponential maximum likelihood estimates on open and closed time distributions of our single HCN and \( I_f \) channel recordings revealed an allosteric multistate gating model comprising at least 4 open and 5 closed states given by the best-fit method.\(^ {23} \)

In atria and ventricles, HCN2 and HCN4 are the dominant HCN isoforms based on Northern blotting and RNase protection, whereas HCN1 is not expressed or is hardly expressed.\(^ {10,12} \) Therefore, we hypothesized that single-channel characteristics of native atrial \( I_f \) would exhibit at least some properties resembling those of recombinant HCN2/HCN4. Although modulation of HCN channels by regulatory \( \beta \)-subunits in native tissue has been proposed,\(^ {31,12} \) unexpectedly, \( I_f \) single-channel characteristics obtained could readily be explained by homomultimerization/heteromultimerization of HCN2 and HCN4. Although this does not exclude modulation by endogenous factors, our observations further support that heteromeric complexes of HCN2 and HCN4 underlie native \( I_f \) in working myocardium.

Most importantly, however, we determined half-maximal activation of single-channel \( I_f \) well within the diastolic voltage range of atrial myocardium. Similar to recombinant HCN channels, activation potentials in our cell-attached recordings were more positive than previously suggested in whole-cell measurements, most likely because we omitted cell dialysis.\(^ {4,5,8,25} \) This observation supports the hypothesis that \( I_f \) might indeed contribute to arrhythmogenesis in diseases characterized by \( I_f \) overexpression such as heart failure, hypertrophy, and atrial fibrillation and indicates a potential therapeutic role of specific \( I_f \) blockers to modify pathological automaticity.\(^ {4,5,8} \) However, further studies exploring whether, in diseased states, \( I_f \) can lead to spontaneous diastolic depolarizations in working myocardium in vivo are necessary.

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References

7. Lai LP, Su MJ, Lin JL, Tsai CH, Lin FY, Chen YS, Hwang JJ, Huang SK, Tseung VY, Lien WP. Measurement of funny current (\( I_f \)) channel mRNA...
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