Letter Regarding Article by Michels et al. “Single-Channel Properties Support a Potential Contribution of Hyperpolarization-Activated Cyclic Nucleotide-Gated Channels and I\(_f\) to Cardiac Arrhythmias”

To the Editor:

“Funny” channels are responsible for generation of pacemaker activity and autonomic modulation of cardiac rate. Because of their low conductance (~1 pS), single f-channels were elusive, and the first (cell-attached) recording published in 1986\(^1\) was achieved thanks to a modification of the patch-clamp technique with 2 pipettes on the same cell, one to apply voltages and one to record single channels. With this arrangement there was no need to subtract capacitive transients in the patch pipette because there were none, which greatly increased the recording resolution. Cell-free inside-out f-channel recording was published later\(^2\) also with a modification (voltage steps delivered via the bath pellet) for improved resolution.

Because our laboratory has been the only one to publish single f-channel data, I read with interest the recent article by Michels and colleagues on single-HCN (hyperpolarization-activated, cyclic nucleotide–gated; the molecular correlates of native pacemaker channels) and human f-channel properties.\(^3\) However, single channels recorded by Michels and coworkers do not appear to be the same as those published previously. One difference is in the conductance, which for native f-channels is nearly 20-fold higher\(^4\) than previously reported.\(^1\) Conductances reported for HCN isoforms are also much higher (~13- to 35-fold). These differences are not compatible with normal variability.

Another substantial difference concerns the current time course. At or around ~90 mV, time constants of activation of I\(_f\), HCN1, -2, and -4 have values close to 1200, 120, 1500 and 3000 ms, respectively.\(^5,6\) Ensemble average current records shown by Michels and collaborators\(^3\) for HCN isoforms and I\(_f\) are, however, flat and do not reveal any time dependence, reflecting an “instantaneous” rather than a time-dependent behavior. The similarity between kinetics of whole-cell and ensemble-average patch current recorded simultaneously from the same cell was one of the distinctive properties of single-channel recordings previously reported,\(^1\) which, together with the direct cAMP-induced activation demonstrated in cell-free mode\(^2\) are characteristic signatures of f-channels.

Another unexplained property, as noted by the authors,\(^3\) is the much more positive activation threshold of single-channel than whole-cell currents; finally, mean first latencies on the order of tens of milliseconds\(^5\) are short compared with the several hundred milliseconds first-latency values previously reported.\(^2\)

It is not clear why the single-channel recordings of Michels and collaborators differ so substantially from previous data. Single-channel time-dependent kinetics mimicking whole-cell kinetics and cAMP sensitivity during direct inside-out patch perfusion should be demonstrated to verify how these recordings relate to the pacemaker current.

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**Response**

The pacemaker current I\(_f\) is a mixed voltage- and ligand-gated cation current with an instantaneous and time-dependent current component. Dr DiFrancesco addresses the important question of why conductance, first latency to open and average currents of the only previous single-channel recordings of I\(_f\) obtained by his group in sinus node cells,\(^1,2\) differ from our recent recordings.\(^3\) Distinct recording techniques may explain these differences. In our experiments, cells remained intact without internal dialysis, whereas previous recordings were performed using the excised-patch technique\(^2\) or whole-cell–driven recordings.\(^1\) Excision of macropatches resulted in negative shifting of the activation curve, which only partly could be explained by the removal of intracellular cAMP, as noticed by DiFrancesco and coworkers,\(^2\) suggesting some thus-far undefined run-down process independent from cAMP on alteration of the solution at the intracellular side. This run-down process seems to shift the activation curve, but also may profoundly alter and prolong first latencies, as previously reported for KAT1 channels.\(^4\) Our data were analyzed in patches containing 1 (most data including average currents) to a maximum of 3 channels as compared with macrochannel patches in previous experiments.\(^1\) Although individual ensemble averages of 1-channel patches did not reliably mimic whole-cell kinetics, consistent with 1-channel recordings of other channels,\(^5\) first latencies were voltage dependent in the expected manner.

Current filtering is distinct: Although our filtering at 2 kHz will not allow recording of very low amplitude currents, previous filtering at 80 to 160 Hz is expected to have a filter rise time of approximately 2 to 4 ms, which does not allow analysis of brief channel openings in the range of milliseconds. Thus, it remains unclear whether f-channels could exhibit 2 distinct conductances (ie, long, low conductance openings and short, higher conductance openings), a hypothesis we are investigating using low-noise recording techniques.\(^6\) Although we did not obtain any shift of the baseline during our 3-s pulses, our experiments cannot rule out a small current component contributed by low conductance channels. Interestingly, using recording techniques similar to ours, an insect homolog of HCN channels (HvCNG) recently displayed a similar conductance of 30 pS.\(^7\) As mentioned by DiFrancesco, a cAMP-induced shift of activation is a characteristic signature of F-channels. Consistent with this typical channel property in our experiments, forskolin significantly shifted the activation curve to more positive potentials (see Figure 2 in our original article\(^8\)) without modification of its shape or the single-channel conductance, which agrees with previous reports.\(^2\) We are thankful for the valuable comments and discussion of DiFrancesco and colleagues, and we look forward to seeing additional interesting results elucidating the function of f-channels.

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