Comments to the Editor

Action Potential Shape Is a Crucial Measure of Cell Type of Stem Cell-Derived Cardiocytes

Cardiac myocytes derived from human-induced pluripotent stem cells (h-iPSCs) are an exciting novel human experimental system that has great promise for use in cardiac drug safety design, testing, and research. Despite the very recent emergence of this technology (1–9), the use of iPSC-derived (iPSCD) cardiocytes in research and drug development has exploded (9–19). Development of safety testing approaches using iPSCD cardiocytes is actively encouraged by the both the FDA (20) and the international consortium that regulates drug safety standards in their recent comprehensive in vitro proarrhythmia assay initiative. Because of the known and suspected limitations in the iPSCD cardiocytes and interpretation of iPSCD cardiocytes data, the comprehensive in vitro proarrhythmia assay initiative states that experimentation on iPSCD cardiocytes should be combined with in silico approaches (20). Considerable debate on what the limitations of this new preparation are, and how to extract the most useful information from it, has been generated. The original Biophysical Letter of Du et al. (21) and the correspondence between Giles and Noble (22) and Kane et al. (23) are an example of this debate.

h-iPSCD cells are used in a variety of preparations and measured using a variety of techniques. The technique of electronic expression of I_{K1} by Bett et al. (8) as employed by our group to distinguish between cell types was discussed extensively in the correspondence between Giles and Noble (22) and Kane et al. (23). The method of Bett et al. (8) uses a dynamic clamp to insert a computer-generated I_{K1} into single iPSCD myocytes. As shown in Fig. 1, this changes the action potential morphology considerably from the spontaneously active case and clearly shows cells with atrial-like and ventricular-like types of action potentials. In contrast, Du et al. (21) used a voltage-sensitive dye, in the presence of blebbistatin, to measure the action potential (shown for comparison in Fig. 1 *E*) in monolayers. The dye measurements of Du et al. (21) somewhat resemble the spontaneous APs recorded under voltage-clamp, but may also reflect some of the limitations of optical measurements of membrane potential. Even minor photodynamic damage associated with Di-8-ANEPPS tends to increase linear background currents that may alter the action potential and change the morphology of both atrial and ventricular myocytes; clearly, the action potentials are more triangular than those measured using other methods (24,25). It is unclear from their example if the methods employed by Du et al. (21) are influencing their ability to clearly distinguish cell type through action potential morphology. Other less damaging and invasive methods can potentially give more detailed information on action potential shape in iPSCD myocytes (24,25). We agree with the observation of Kane et al. (23) that more studies are needed "involving genetically encoded or more quantitative and less toxic ratiometric dyes".

Careful scrutiny of data is always important and we read with great interest the reanalysis of one of the figures looking at the distribution of cell types within the correspondence of Kane et al. (23). Fig. 1 *B* from Kane et al. (23) shows a fit of a hyperbolic equation designed to pass through our data, which Kane et al. (23) suggests is indicative of a potentially continuous distribution of cell types. Our interpretation is very different. We view the need to use such a sharply curved line in this fit showing that we have indeed two separated two distinct populations. This is further reinforced by the rather poor fit of the curve to the ventricular-like cell data. At the inflection point, there may indeed be some overlap in AP characteristics between types. In years of working with acutely isolated adult myocytes, it is clear that even for freshly isolated adult heart myocytes there is a significant overlap in shape between ventricular and atrial cells.

In their reanalysis of our data, Kane et al. (23) comment heavily on variability and "argue that action potential duration is fairly homogeneously distributed, even after corrections for I_{K1}". We appreciate the chance to comment directly on this misconception about the data in the article of Bett et al. (8). In Bett et al. (8), we used a single magnitude and representation of I_{K1} for every cell. I_{K1} was not cell-specific, and was not matched to cell type or capacitance. The result is that for ventricular-like cells, a very low I_{K1} current density was used relative to that found physiologically in adult ventricular myocytes. Conversely, a somewhat high density was used for atrial-like cells. This is due both to the larger capacitance of the ventricular-like types and the much higher I_{K1} density in adult ventricular cells relative to adult atrial myocytes. This explains why, under the conditions of Bett et al. (8), ventricular-like cells have durations of >1 s. Lack of cell-specific tuning of I_{K1}

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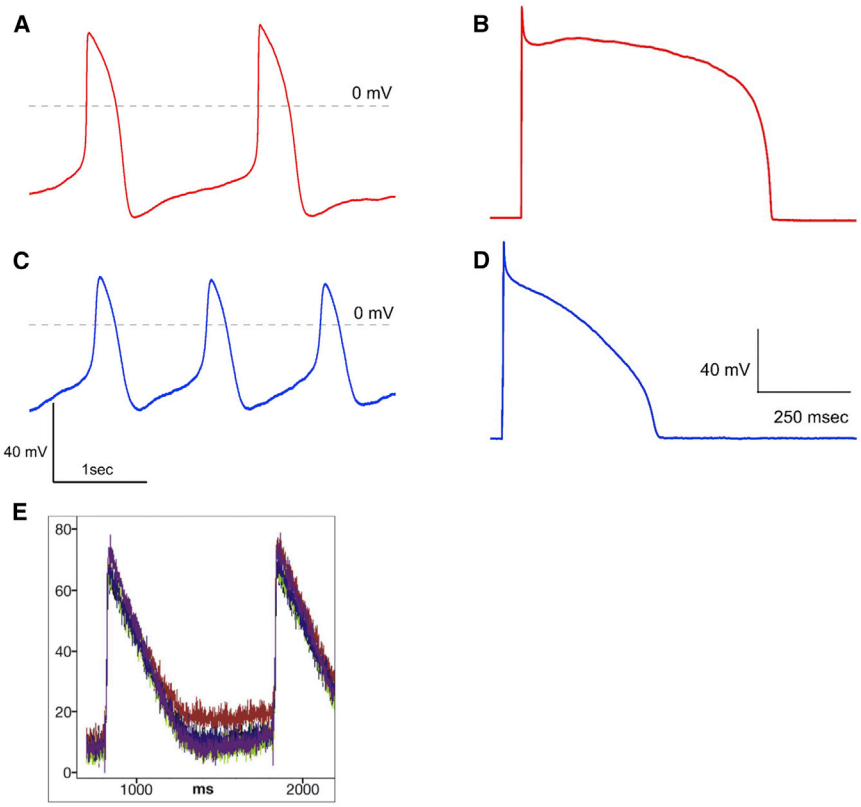

FIGURE 1 Action potential morphology. (*A*) Spontaneous action potentials from ventricular type h-iPSCD cardiac cell recorded using the patch-clamp technique. (*B*) Stimulated action potentials from the same cell with electronic expression of I_{K1}. (*C*) Spontaneous action potentials from an atrial type h-iPSCD cardiac cell recorded using the patch-clamp technique. (*D*) Stimulated action potentials from the same cell with electronic expression of I_{K1}. (*E*) Representative monolayer action potentials recorded using optical techniques from the article of Du et al. (21).

also explains a large portion of the cell variability in the article of Bett et al. (8). Both adult cardiac ventricular myocytes as well as iPSCD cells vary significantly in capacitance. Because a one-size-fits-all approach was used in that article, the current density (in pA/pFd) of I_{K1} also varied accordingly and AP duration is strongly and nonlinearly sensitive to I_{K1} density (26–28). In future studies looking at cell physiology, it will be useful to normalize the magnitude of electronic expression of I_{K1} to provide a uniform current density rather than an identical per cell current. In this study, the analysis of Kane et al. (23) is inappropriate.

Kane et al. (23) also transformed data from Bett et al. (8), as shown in Fig. 1 *D*. They assert that these data do "not cluster into two subpopulations and there is no relationship between this distribution and the ventricular label assigned in Fig. 1 A". We disagree; a preliminary cluster analysis of our data using a K-means test (29) for $K = 2$ suggests two separate groups, portioning roughly along the lines of our criterion and with a $p < 0.001$ of being random. The difference between APD30 and APD90 covers a range where I_{K1} is more active than at the plateau. Therefore, the absolute magnitude of APD30-APD90 is an inappropriate measure of action potential shape differences and is a misinterpretation of the data from the article of Bett et al. (8). The data transformation of Kane et al. (23) in Fig. 1 *D* still shows clear separation of components. Essentially, all of the ventricular-like points lie above the atrial-like points on this *x*,*y* plot. This difference clearly shows that there is indeed a shape difference and that this occurs in early repolarization, where synthetic I_{K1} is relatively unimportant.

Human iPSCD cardiocyte technology is only a few years old and being explored in many directions, using many approaches. Very basic questions are being addressed, including: do chamber-specific cell types exist in cultured iPSCD cardiocyte preparations? Can they be identified quickly? How can they be identified? These are very important questions, and will not be answered in a single article. We agree with both groups that more experimental evidence to support the idea that iPSC-CMs spontaneously form distinct, chamber-specific subtypes is needed. Bett et al. (8) shows that in the presence of electronic expression of I_{K1}, action potentials can be separated into distinct populations and that the morphology of these action potentials is consistent with an atrium versus ventricle distinction. The next step is to demonstrate that this sorting based on morphology provides consistent data with other chamber-specific cellular properties. Preliminary data from our laboratory (30) presented in abstract form at this year's Biophysical Society Meeting suggests that this is the case; distinct action potential morphologies have underlying channel current properties consistent with an atrial versus ventricle phenotype.

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REFERENCES

1. Itzhaki, I., L. Maizels, …, L. Gepstein. 2011. Modelling the long QT syndrome with induced pluripotent stem cells. *Nature.* 471:225–229.
2. Narazaki, G., H. Uosaki, …, J. K. Yamashita. 2008. Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. *Circulation.* 118:498–506.
3. Kuzmenkin, A., H. Liang, …, J. Hescheler. 2009. Functional characterization of cardiomyocytes derived from murine induced pluripotent stem cells in vitro. *FASEB J.* 23:4168–4180.
4. Moretti, A., M. Bellin, …, K. L. Laugwitz. 2010. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N. Engl. J. Med.* 363:1397–1409.
5. Liu, J., P. J. Verma, …, A. Pebay. 2010. Generation of induced pluripotent stem cell lines from Friedreich ataxia patients. *Stem Cell Rev.* 7:703–713.
6. Gai, H., E. L. Leung, …, Y. Ma. 2009. Generation and characterization of functional cardiomyocytes using induced pluripotent stem cells derived from human fibroblasts. *Cell Biol. Int.* 33:1184–1193.
7. Tanaka, T., S. Tohyama, …, K. Fukuda. 2009. In vitro pharmacologic testing using human induced pluripotent stem cell-derived cardiomyocytes. *Biochem. Biophys. Res. Commun.* 385:497–502.
8. Bett, G. C., A. D. Kaplan, …, R. L. Rasmusson. 2013. Electronic "expression" of the inward rectifier in cardiocytes derived from human-induced pluripotent stem cells. *Heart Rhythm.* 10:1903–1910.
9. Parikh, A., D. Patel, …, G. Salama. 2013. Relaxin suppresses atrial fibrillation by reversing fibrosis and myocyte hypertrophy and increasing conduction velocity and sodium current in spontaneously hypertensive rat hearts. *Circ. Res.* 113:313–321.
10. Knollmann, B. C. 2013. Induced pluripotent stem cell-derived cardiomyocytes: boutique science or valuable arrhythmia model? *Circ. Res.* 112:969–976.
11. Sinnecker, D., A. Goedel, …, A. Moretti. 2013. Induced pluripotent stem cell-derived cardiomyocytes: a versatile tool for arrhythmia research. *Circ. Res.* 112:961–968.
12. Priori, S. G., C. Napolitano, …, G. Condorelli. 2013. Induced pluripotent stem cell-derived cardiomyocytes in studies of inherited arrhythmias. *J. Clin. Invest.* 123:84–91.
13. Abbott, G. W. 2013. KCNE genetics and pharmacogenomics in cardiac arrhythmias: much ado about nothing? *Expert Rev. Clinical Pharmacol.* 6:49–60.
14. Friedrichs, S., D. Malan, and P. Sasse. 2013. Modeling long QT syndromes using induced pluripotent stem cells: current progress and future challenges. *Trends Cardiovasc. Med.* 23:91–98.
15. Kraushaar, U., T. Meyer, …, E. Guenther. 2012. Cardiac safety pharmacology: from human Ether-á-gogo related gene channel block towards induced pluripotent stem cell based disease models. *Expert Opin. Drug Saf.* 11:285–298.
16. Lu, T. Y., and L. Yang. 2011. Uses of cardiomyocytes generated from induced pluripotent stem cells. *Stem Cell Res. Therapy.* 2:44.
17. Liang, P., F. Lan, …, J. C. Wu. 2013. Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation.* 127:1677–1691.
18. Gibson, J. K., Y. Yue, …, R. Numann. 2014. Human stem cell-derived cardiomyocytes detect drug-mediated changes in action potentials and ion currents. *J. Pharmacol. Toxicol. Methods.* 70:255–267.
19. Nozaki, Y., Y. Honda, …, H. Funabashi. 2014. Availability of human induced pluripotent stem cell-derived cardiomyocytes in assessment of drug potential for QT prolongation. *Toxicol. Appl. Pharmacol.* 278:72–77.
20. Sager, P. T., G. Gintant, …, N. Stockbridge. 2014. Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium. *Am. Heart J.* 167:292–300.
21. Du, D. T., N. Hellen, …, C. M. Terracciano. 2015. Action potential morphology of human induced pluripotent stem cell-derived cardiomyocytes does not predict cardiac chamber specificity and is dependent on cell density. *Biophys. J.* 108:1–4.
22. Giles, W. R., and D. Noble. 2016. Rigorous Phenotyping of Cardiac iPSC Preparations Requires Knowledge of Their Resting Potential(s). *Biophys. J.* 110:278–280.
23. Kane, C., D. T. M. Du, N. Hellen, and C. M. Terracino. 2016. The fallacy of assigning chamber specificity to iPSC cardiac myocytes from action potential morphology. *Biophys. J.* 110:281–283.
24. Shinnawi, R., I. Huber, …, L. Gepstein. 2015. Monitoring human-induced pluripotent stem cell-derived cardiomyocytes with genetically encoded calcium and voltage fluorescent reporters. *Stem Cell Rep.* 5:582–596.
25. Kim, J. J., L. Yang, …, G. Salama. 2015. Mechanism of automaticity in cardiomyocytes derived from human induced pluripotent stem cells. *J. Mol. Cell. Cardiol.* 81:81–93.
26. Cordeiro, J. M., T. Zeina, …, B. K. Panama. 2015. Regional variation of the inwardly rectifying potassium current in the canine heart and the contributions to differences in action potential repolarization. *J. Mol. Cell. Cardiol.* 84:52–60.
27. Lieu, D. K., J. D. Fu, …, R. A. Li. 2013. Mechanism-based facilitated maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ. Arrhythm. Electrophysiol.* 6:191–201.
28. Meijer van Putten, R. M., I. Mengarelli, …, R. Wilders. 2015. Ion channelopathies in human induced pluripotent stem cell derived cardiomyocytes: a dynamic clamp study with virtual I_{K1}. *Front. Physiol.* 6:7.
29. Remm, K., and T. Kelviste. 2014. An online calculator for spatial data and its applications. *Comput. Ecol. Softw.* 4:22–34.
30. Kaplan, A. D., G. C. L. Bett, and R. L. Rasmusson. 2015. Electronic expression of I_{K1} in human induced pluripotent stem cell derived cardiocytes reveals atrial versus ventricular specific properties. *Biophys. J.* 108:112a.