Reviewer’s report

**Title:** Epinephrine Reversed High-Concentration Bupivacaine-Induced Inhibition of Calcium Channels and Transient Outward Potassium Current Channels, but Not on Sodium Channel in Ventricular Myocytes of Rats

**Version:** 2  
**Date:** 24 October 2014

**Reviewer:** Stuart Forman

**Reviewer’s report:**

The manuscript describes electrophysiological studies of cationic currents in cardiac ventricular myocytes under conditions that emulate those during bupivacaine-induced cardiac arrest and epinephrine resuscitation in an isolated perfused heart model. While others have previously performed similar experiments on isolated currents, there are contradictory results in the literature, providing adequate justification for these studies.

The data is presented in a clear manner and the results appear to support most of the conclusions. However, it is unclear why peak currents rather than area under the curve is measured. Some of the methods are described with insufficient clarity and detail and the English language usage in the methods section is not as clear as in other sections. These issues must be addressed before the results can be judged to be reliable. The statistical approach also needs to be amended to account for multiple comparisons within each data set.

The methods indicate humane treatment of animals and I found no ethical breaches.

**Major compulsory revisions:**

**Methods:**

1) Pg 5: It is unclear why the isolated heart preparation is described, unless it was done to facilitate distribution of collagenase via the circulation. If the same hearts were used for physiological experiments with bupivacaine and epinephrine prior to treatment and isolation of myocytes for the electrophysiological current studies, that needs to be clarified.

2) Pg. 6: Please describe the external salt solution used for electrophysiology experiments. Only the internal pipette solutions are described.

3) The descriptions of voltage pulse protocols used for stimulation of various currents is confusing and needs to be re-written by an English-speaking individual who is also familiar with cellular electrophysiology. It would be very helpful if the voltage pulse protocols were illustrated along with the sample recordings in Figs 1, 3, and 5.

4) Please clarify that Ito is a transient outward K+ current at its first use in the
manuscript.

5) It is unclear whether the experiments were performed using bath perfusion or single-cell “microperfusion.” Also, I don’t understand the meaning of performing recordings “immediately after perfusion.” Was perfusion halted during recordings? Was it simply bath exchange of one solution for another? Again, clear English from someone who understands the technical aspects of the experiments is needed.

6) If recording frequency was 0.2Hz (every 5 seconds), does this apply to the period between sweeps at different voltage steps in I-V experiments? If so, the experiments must have lasted longer than 10 seconds, and I don’t understand what recording for 10 seconds means. The authors must clearly describe how external solutions were changed, the relative timing of solution exchange and experiments, and how many times the experiments were performed on each cell. Also, the authors must state whether or not the currents are stable over longer time periods after solution exchange, or was there only one recording from each cell at each experimental time-point?

7) Page 8: Each current in Table 1 is compared to two others, and a total of 3 pairwise comparisons are made for each set of current measurements. Therefore, using the Bonferroni correction, the P value for statistical significance when using paired t-tests should be $0.05/3 = 0.017$. This impacts the significance of one outcome of the study: comparison of T1 vs T0 for Ito.

8) Results: It is unclear why the authors only analyzed peak currents, when the currents are inactivating and it is possible that the drugs affect inactivation rates. For example, it appears that Ito inactivation is reduced by bupivicaine, resulting in less inhibition of steady-state currents compared with peak currents (Fig 5). Analysis should be done on the area under the curve (AUC) for comparison with peak currents.

9) Figures 1, 3, and 5 should include voltage pulse illustrations.

Minor Essential Revisions:

1) I am confused by the term “nominally Ca2+ free” in reference to a Tyrode solution that contains 1.8 mM CaCl2.

2) The manufacturer that supplied electrophysiological equipment and Pulse 8.0 software is HEKA—please correct spelling.

3) Page 7: Please clarify that ICa-L is an inward current and detail the external Ca2+ concentration. Otherwise, the use of EGTA in the internal solution would inhibit outward Ca currents.

4) Examining Figures 5 and 6 seems to show a conflict. The example in Fig 5 shows that the bupivicaine + epi T2 Ito peak current is smaller than the controls (T0), yet Fig 6 suggests that that peak currents in the presence of bupivicaine + epi are larger than control peaks. Which is correct?
5) Table 1 appears to be entirely redundant with results given in the text. This seems unnecessary.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests