

Burleigh®

Micromanipulators • In Control

Simultaneous Whole-Cell Patch-Clamp of Cardiac Ventricular Myocytes and Confocal Calcium Transient Imaging

Challenge

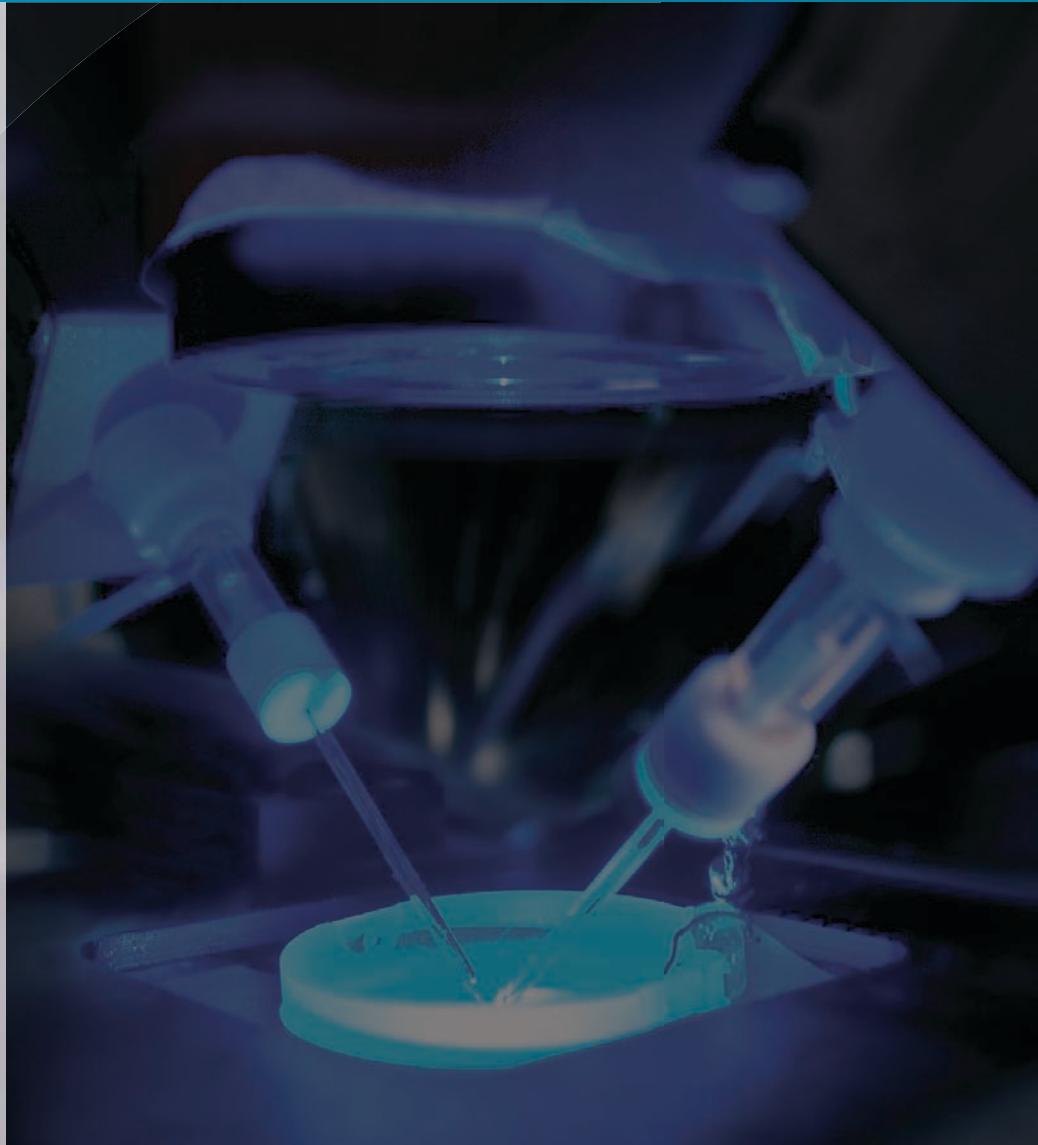
Pipette stability when acquiring voltage clamp recordings simultaneously with confocal microscopy

Solution

Burleigh® micromanipulator providing a stable electrophysiology set-up

Benefit

A stable patch-clamp experiment leading to drift-free and accurate recordings



Repolarization or T wave alternans (TWA) is an electrocardiographic (ECG) phenomenon characterized by a beat-to-beat alternation of the amplitude, morphology, and/or polarity of the T wave. TWA can occur under a variety of pathophysiological conditions such as acquired and congenital long QT syndromes⁷, heart failure⁴ and cardiac ischemia⁵ producing the conditions for the initiation of arrhythmias (arrhythmogenic substrate).

The Ca^{2+} transient that controls cell shortening during excitation-contraction (EC) coupling is a dynamic process that is controlled by Ca^{2+} influx and release, as well as Ca^{2+} reuptake and extrusion. A number of studies have highlighted the regional differences in mechanical shortening and $[\text{Ca}^{2+}]_i$ cycling across the wall of the left ventricle. This study examines regional differences in the rate-dependence of Ca^{2+} transient characteristics under rapid pacing conditions that give rise to APD (action potential duration) and associated T wave alternans.

Whole-cell patch-clamp of cardiac myocytes requires a stable micromanipulator for accurate recordings. Mechanical stability of the pipette is essential when the voltage clamp recordings are simultaneously acquired with confocal microscopy. Any drift or vibrations arising from the micromanipulator may lead to distortions in the fluorescence signal and artifactual recordings. The Burleigh® PCS-5000 Micromanipulator has been crucial in providing high quality recordings by helping to overcome these problems of mechanical instability.

Methods

Myocytes from epicardial (EPI), endocardial (ENDO) and midmyocardial (MID) regions were prepared from canine hearts using techniques previously described^{1,2}. The acetoxymethylester (AM) form of Fluo-4 was used. About 1.5 ml of cell suspension containing either EPI, ENDO,

or MID cells were added to the Fluo-4/AM (dissolved in 20% F-127 pluronic in DMSO, final concentration approximately 15 μM) for 20 minutes at room temperature as previously described³. All confocal experiments were performed with a laser-scanning confocal microscope. Fluo-4 loaded myocytes were placed in a perfusion chamber, excited at 488 nm fluorescence emission detected via a 520 nm band-pass filter and photomultiplier tube.

Simultaneous voltage clamp and confocal recordings of Fluo-4 loaded ventricular cells were made. Pipettes were pulled using a gravity puller (model PP-830, Narashige, Japan). Pipettes were filled with internal solution and placed in a pipette holder and a Burleigh® micromanipulator (model PCS-5000, Burleigh®, Lumen Dynamics Group Inc.). Ionic currents were recorded with an Axoclamp 1D amplifier (Axon Instruments) and series resistance errors were compensated 65-70%. Recordings occurred about 4 minutes after membrane rupture and experiments lasted about 5 minutes thereafter. Membrane currents were recorded and analyzed using pClamp 9.0 software (Axon Instruments). All confocal experiments were performed at 36°C.

Results

Figure 1 shows representative ion channel currents and corresponding Ca^{2+} transients recorded from Fluo-4 loaded EPI, MID and ENDO cells during a 300 msec pulse. Prior to the application of the test pulse, 5 square pre-pulses were applied at a BCL=500 msec to maintain a uniform SR (sarcoplasmic reticulum) Ca^{2+} content⁶. A large inward Na^+ current was recorded in all 3 cell types in response to a depolarization to +20 mV (I_{Na} truncated for clarity). A prominent I_{to} was also observed in both EPI and MID cells (Figure 1, upper traces). The corresponding confocal xt line scan shows a rapid rise in Ca^{2+} throughout the cell upon depolarization (Figure 1, lower traces).

Discussion

Our data demonstrated that cells exhibiting a slower decay of the Ca^{2+} transient were more susceptible to the development of alternans during faster pacing conditions. We also demonstrated that in cardiac cells lacking a well organized SR (neonate ventricular cells), alternans were rarely observed. This data coupled with the observation that alternans is abolished when SR function is impaired by treatment with ryanodine⁷ provide further evidence in support of the hypothesis that SR calcium cycling is a major component in the development of alternans.

Mechanical stability of the pipette is essential when performing simultaneous voltage clamp and confocal microscopy recordings. Drift or vibrations arising from the micromanipulator may lead to distortions in the fluorescence signal and artifactual recordings. In a typical experiment using a Burleigh[®] manipulator, drift is less than 1 μm per hour. In this experiment, the Burleigh[®] PCS-5000 Micromanipulator was instrumental in ensuring highest quality recordings by minimizing mechanical instability caused by disruptions in patching. In addition, the precision and control provided by the Burleigh[®] manipulator enabled an easy and highly stable experimental set-up resulting in acquisition of reliable electrical and confocal data.

Summary

Mechanical stability of the pipette is essential when the voltage clamp recordings are simultaneously acquired with confocal microscopy. Any drift or vibrations arising from the micromanipulator may lead to distortions in the fluorescence signal and artifactual recordings. The use of

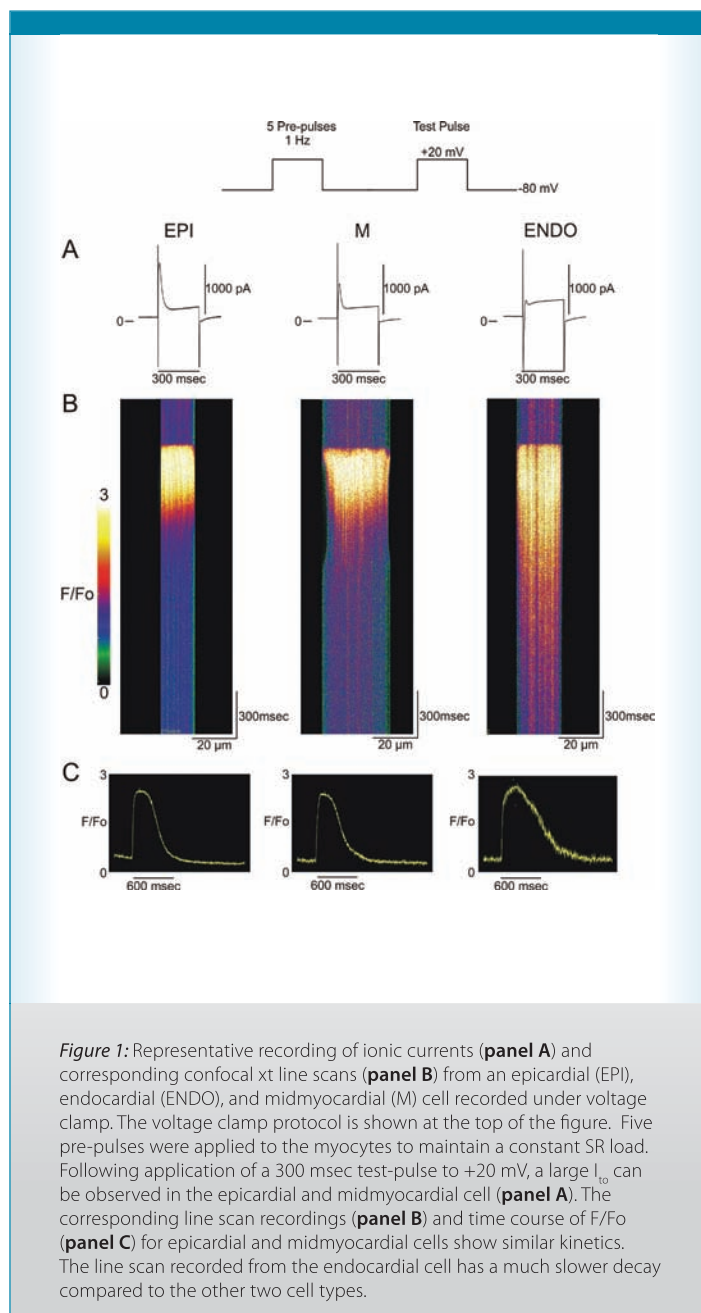


Figure 1: Representative recording of ionic currents (**panel A**) and corresponding confocal xt line scans (**panel B**) from an epicardial (EPI), endocardial (ENDO), and midmyocardial (M) cell recorded under voltage clamp. The voltage clamp protocol is shown at the top of the figure. Five pre-pulses were applied to the myocytes to maintain a constant SR load. Following application of a 300 msec test-pulse to +20 mV, a large I_{to} can be observed in the epicardial and midmyocardial cell (**panel A**). The corresponding line scan recordings (**panel B**) and time course of F/Fo (**panel C**) for epicardial and midmyocardial cells show similar kinetics. The line scan recorded from the endocardial cell has a much slower decay compared to the other two cell types.

a stable micromanipulator is crucial for whole-cell patch-clamp experimental accuracy. The Burleigh[®] micromanipulator provides maximum stability for minimal vibration and drift-free experiments leading to accurate patch recordings.

References

1. Cordeiro JM, Greene L, Heilmann C, Antzelevitch D, & Antzelevitch C (2004). Transmural heterogeneity of calcium activity and mechanical function in the canine left ventricle. *Am J Physiol Heart Circ Physiol* **286**, H1471-H1479.
2. Cordeiro JM, Malone JE, Di Diego JM, Scornik FS, Aistrup GL, Antzelevitch C, & Wasserstrom JA (2007). Cellular and subcellular alternans in the canine left ventricle. *Am J Physiol Heart Circ Physiol* **293**: H3506-H3516.
3. Cordeiro JM, Spitzer KW, Giles WR, Ershler PE, Cannell MB, & Bridge JH (2001). Location of the initiation site of calcium transients and sparks in rabbit heart Purkinje cells. *J Physiol* **531**, 301-314.
4. Klingenhoben T, Zabel M, D'Agostino RB, Cohen RJ, & Hohnloser SH (2000). Predictive value of T-wave alternans for arrhythmic events in patients with congestive heart failure. *Lancet* **356**, 651-652.
5. Lakireddy V, Baweja P, Syed A, Bub G, Boutjdir M, & El-Sherif N (2005). Contrasting effects of ischemia on the kinetics of membrane voltage and intracellular calcium transient underlie electrical alternans. *Am J Physiol Heart Circ Physiol* **288**, H400-H407.
6. Litwin SE, Li J, & Bridge JH (1998). Na-Ca exchange and the trigger for sarcoplasmic reticulum Ca release: studies in adult rabbit ventricular myocytes. *Biophys J* **75**, 359-371.
7. Shimizu W & Antzelevitch C (1999). Cellular and ionic basis for T-wave alternans under Long QT conditions. *Circulation* **99**, 1499-1507.

For more information on the Burleigh® Micromanipulators, please visit <http://www.LDGI-Burleigh.com/products-manipulators.php>. If you would like to share your research involving the use of a Burleigh® system in a publication of this type, please contact Dr. Kavita Aswani at Kavita.Aswani@LDGI.com. For more information on Burleigh® Products and their Applications, please visit www.LDGI-Burleigh.com



LUMEN DYNAMICS
PUTTING YOU IN CONTROL

2260 Argentia Road,
Mississauga, Ontario,
L5N 6H7 CANADA

www.LDGI-Burleigh.com

Telephone: +1 905 821-2600
Toll Free (USA and Canada): +1 800 668-8752
Facsimile: +1 905 821-2055

Burleigh@LDGI.com