Detection and Modulation of Cardiomyocyte Calcium Oscillations Using the FDSS6000

Introduction

Current methodology to detect calcium oscillations in mouse fetal cardiomyocytes uses fluorescence microscopy to image cells plated on cover slips or Petri dishes. Using this model cells are exposed to putative cardiomodulators to investigate their pharmacological or toxicological properties. We report a methodology to transfer this model to high throughput screening using the FDSS6000.

Wells show a baseline calcium oscillation (pulse) rate of either about 1/sec or appear quiescent. In both well types the agonist Isoproterenol increased pulse rate. In the latter wells we show the antagonist Propranolol blocks Isoproterenol agonism.

Materials and Methods

Briefly, fetal mouse cardiomyocytes collected and purified to small aggregates (30 to 100 clumps per well). Cells were seeded into collagen-coated 96 wells and noted to attach and contract in unison within two to three days. Typically, clumps contracted vigorously for three to seven days at 20-40 beats per minute, becoming quiescent with prolonged culture. Cells were dye loaded with Fluo-4 AM (2.5 μM final). Following a 1 h incubation at 37 °C cells were washed twice.

FDSS6000 was configured to integrate signal for 69 ms with a 300 ms interval between readings. After 15 sec of readings 50 μL of reagent was added to cells covered with 100 μL buffer, at 100 μL/s, 4 mm above cell layer. Signal was collected for approximately one minute following fluid addition. Data is reported as a pseudoratio of Reading X/ Reading #1.

Results and Discussion

An initial reading of the 96 well plate revealed wells having one of two types of baseline signals: Quiescent or pulsing. To two wells with similar baseline pulse rates and amplitudes we added the agonist 75 μM Isoproterenol (Figure 1). There was over a 2 fold increase in pulse rate in both wells while the mean amplitude decreased to nearly half baseline.

Next we tested two quiescent wells for activity using 75 μM Isoproterenol. Interestingly, we discovered the wells were sensitive to agonism (Figure 2, arrow #1) as we could detect an increase in amplitude and pulse rate. Following ten minutes of Isoproterenol exposure we treated one well to the b-antagonist Propranolol, the other to buffer only (Figure 2, arrow #2).

The latter showed an increase in amplitude 2.7 fold; by contrast antagonist exposure decreased amplitude by half. Taken together, these results indicate quiescent cells are stimulated with agonists and inhibited with antagonists, in a quantifiable manner.

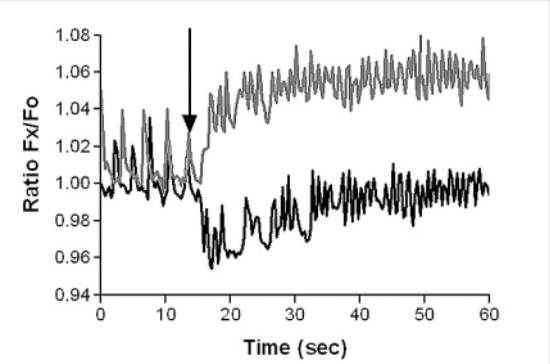

Fig. 1: Effect of Isoproterenol on pulse rate and amplitude. Cells with mean population pulse rates of 0.33/s, 0.04 amplitude were agonized using 75 μM Isoproterenol (arrow). Pulse rates increased an average of 2.3 fold, mean amplitudes decreased nearly in half. For the grey trace, reading 8 was subtracted from all readings so both baseline signals appear equivalent.

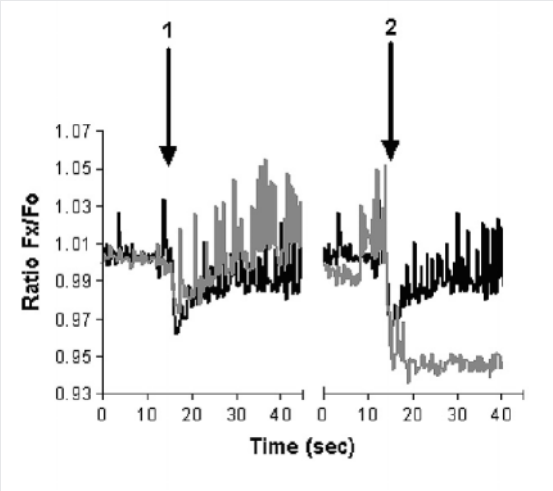

Fig. 2: Effect of β-agonist and β-antagonist on cell populations with quiescent background signals. Cell populations with apparently no pulse signal or amplitude were agonized using 75 μM Isoproterenol (arrow #1). Both mean signal pulse rate and amplitude increased. Ten minutes later either buffer (black trace) or 75 μM Propranolol (grey trace) was added to cells (arrow #2). With buffer addition mean amplitude increased 2.7 fold, with Propranolol mean amplitude decreased to half.

Summary

This report demonstrates the evolution of the mouse fetal cardiomyocyte model from single cell calcium oscillation measurement methodology to the high throughput screening platform FDSS6000.

HAMAMATSU PHOTONICS K.K. **www.hamamatsu.com**

HAMAMATSU PHOTONICS K.K., Systems Division
812 Joko-cho, Higashi-ku, Hamamatsu City, 431-3196, Japan, Telephone: (81)53-431-0124, Fax: (81)53-435-1574, E-mail: export@sys.hpk.co.jp

U.S.A.: Hamamatsu Corporation: 360 Foothill Road, Bridgewater. N.J. 08807-0910, U.S.A., Telephone: (1)908-231-0960, Fax: (1)908-231-1218 E-mail: usa@hamamatsu.com
Germany: Hamamatsu Photonics Deutschland GmbH: Arzbergerstr. 10, D-82211 Herrsching am Ammersee, Germany, Telephone: (49)8152-375-0, Fax: (49)8152-2658 E-mail: info@hamamatsu.de
France: Hamamatsu Photonics France S.A.R.L.: 19, Rue du Saule Trapu, Parc du Moulin de Massy, 91882 Massy Cedex, France, Telephone: (33)1 69 53 71 00, Fax: (33)1 69 53 71 10 E-mail: infos@hamamatsu.fr
United Kingdom: Hamamatsu Photonics UK Limited: 2 Howard Court, 10 Tewin Road, Welwyn Garden City, Hertfordshire AL7 1BW, United Kingdom, Telephone: (44)1707-294888, Fax: (44)1707-325777 E-mail: info@hamamatsu.co.uk
North Europe: Hamamatsu Photonics Norden AB: Torshamnsgatan 35 SE-164 40 Kista, Sweden, Telephone: (46)8-509-031-00, Fax: (46)8-509-031-01 E-mail: info@hamamatsu.se
Italy: Hamamatsu Photonics Italia S.r.l.: Strada della Moia, 1 int. 6, 20020 Arese (Milano), Italy, Telephone: (39)02-93581733, Fax: (39)02-93581741 E-mail: info@hamamatsu.it
China: Hamamatsu Photonics (China) Co., Ltd.: B1201 Jiaming Center, No.27 Dongsanhuan Beilu, Chaoyang District, Beijing 100020, China, Telephone: (86)10-6586-6006, Fax: (86)10-6586-2866 E-mail: hpc@hamamatsu.com.cn

Cat. No. SBIS0034E02
NOV/2013 IP
Created in Japan (PDF)