

**ELECTROPHYSIOLOGICAL PROPERTIES
OF THE RAINBOW TROUT CARDIAC MYOCYTES
MAINTAINED IN SERUM-FREE PRIMARY CULTURE**

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EXTENDED ABSTRACT ONLY -- DO NOT CITE

Introduction

The sarcolemmal ion currents regulate excitation and contraction of the cardiac myocyte and are intimately involved in cardiac adaptation to altered functional demand. The expression of sarcolemmal ion currents is changed in hypertrophied and diseased heart as well as in thermally acclimated animals. The underlying regulatory mechanisms are, however, poorly known. The mechanisms for altered channel expression are best examined in simple systems where regulatory factors can be experimentally controlled. Such a system could be a serum-free primary culture of cardiac myocytes, provided that the electrophysiological properties of the myocytes remain stable or change in a predictable and consistent manner during culture. The aim of the present work was to develop a serum-free primary culture of fish cardiac myocytes which could be used for studies on the regulation of ion channel/current expression in fish cardiac myocytes under diverse environmental stresses.

Methods

Myocytes were isolated from the ventricle of rainbow trout (acclimated at 17-18°C) using collagenase and trypsin (Vornanen, 1998). Cell cultures were prepared with the so-called rapid attachment method where the cells retain their rod-shaped morphology and contractile quiescence for a relatively long time (Piper et al., 1982). Myocytes

were allowed to attach on glass cover slips in plastic petri-dishes and were cultured at +17°C (19% O₂, 2% CO₂) for a maximum of 10 days. Culture medium (DMEM, pH 7.6) was supplemented with 12 mM NaHCO₃, antibiotics (streptomycin and penicillin 100 U ml⁻¹) and 30 mM ARA-C. No serum was added. Membrane potentials and ion currents were measured from freshly isolated cells and cultured cardiac cells using the whole-cell patch-clamp technique at 17 °C.

Results

The cells retained their cross-striated, rod-shaped morphology and most of the cells did not show any spontaneous contractile activity during culture. The whole-cell capacitance remained constant, suggesting that the cell size does not change under serum-free culture conditions.

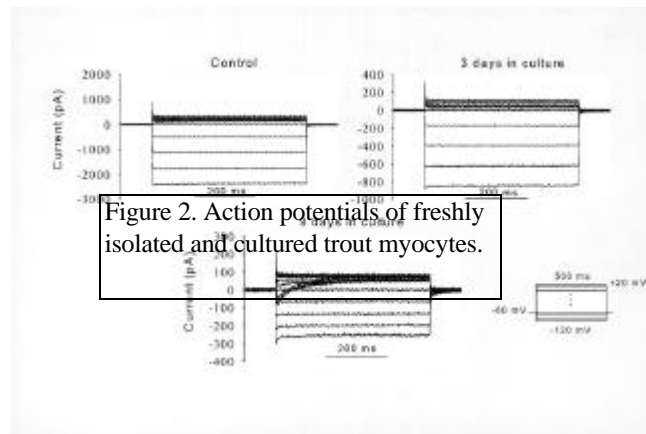
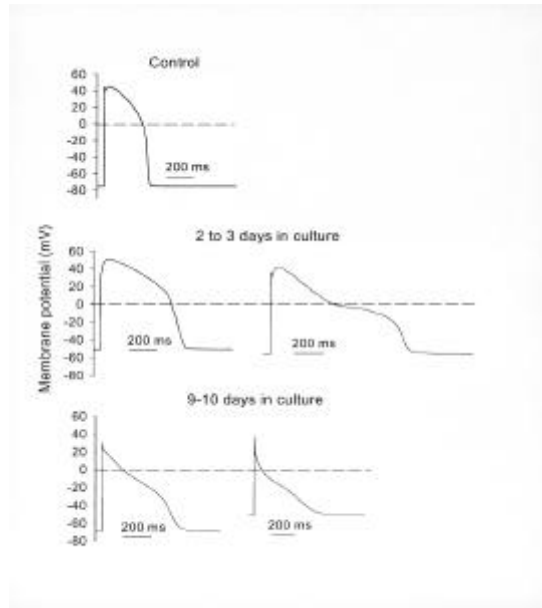


Figure 1. Representative recordings of inwardly rectifying K⁺ current (I_{K1}) in freshly isolated and cultured cardiac cells. Note the presence of the delayed rectifier current (I_{Kr}) in the 9-day cultured myocyte.



There were some prominent changes in the density of sarcolemmal cation currents during culture. The density of the Na^+ current (I_{Na}) decreased progressively from the level of freshly isolated myocytes ($192 \pm 24 \text{ pA pF}^{-1}$; 100%) to ~50% and ~25% in cells cultured for 2-3 and 8-10 days, respectively. The density of the inwardly rectifying K^+ current (I_{K1}) decreased strongly during culture, with the most dramatic change occurring within the first 2 or 3 days. In contrast, the delayed rectifier K^+ current (I_{Kr}) was hardly seen in freshly isolated cells, but was clearly present after 8-10 days of culture. The density of L-type Ca^{2+} current decreased 36% during the first 2 or 3 days of culture, but recovered to the initial control level of the freshly isolated myocytes ($3.0 \pm 0.2 \text{ pA pF}^{-1}$) after 8-10 days.

The changes in sarcolemmal ion currents were reflected in resting potential (RP) and in the shape of action potential (AP) (Fig. 2). RP was less in cultured cells than in freshly isolated cells probably due to the depression of I_{K1} . AP overshoot of the cultured cells was small because of the depolarised RP and low density of I_{Na} . AP

durations at 90% repolarisation level (APD_{90}) were longer in culture cells than in freshly isolated cells. This could be due to stronger depression of I_{K1} relative to I_{Ca} during culture. Furthermore, the plateau duration was markedly shortened in 8-10-day cultured cells, probably due to the development of the rapid component of the delayed rectifier (I_{Kr}) during later phases of the culture.

Conclusions

The densities of I_{Na} and I_{K1} were permanently depressed during culture, whereas the density of I_{Ca} was only transiently down-regulated. The changes in the density of I_{K1} and I_{Na} , are surprisingly similar to those found in serum-free culture of the mammalian ventricular myocytes (Mitcheson et al., 1996; they did not examine I_{Na}). The densities of inwardly rectifying K^+ current, I_{K1} and the delayed rectifier current, I_{Kr} were changed in opposite manner during culture which is reminiscent with the expression of these currents in thermally acclimated fish. Therefore, it seems probable that the expression of I_{K1} and I_{Kr} is under a mutual regulatory pathway.

The present findings indicate that fish cardiac myocytes can be kept in serum-free primary culture for at least 10 days at +17EC. The electrophysiological characteristics of the myocytes change markedly but in a well-predicted and consistent manner during culture. The serum-free culture of the fish cardiac myocytes is a promising preparation which can be used in the future to clarify how the expression of sarcolemmal cation currents is regulated by temperature and other environmental stresses.

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