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RESEARCH ARTICLE

Cardiac Excitation and Contraction

Modifying the electrical excitability of cardiomyocytes in rainbow trout (*Oncorhynchus mykiss*) through exercise training

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Abstract

Exercise training is known to improve the function of the heart in fish. However, at the cellular level, the mechanisms for improvements are still largely unknown. Therefore, we aimed to investigate the impact of exercise training on electric excitation of cardiac contraction in ventricular myocytes of rainbow trout (*Oncorhynchus mykiss*) through the investigation of sodium (I_{Na}), L-type calcium (I_{CaL}), delayed and inward rectifier potassium (I_{Kr} and I_{K1}) currents and action potential (AP) characteristics. The fish were divided into untrained (control) and trained groups. Control fish were kept in standard holding tanks with a water flow rate of 0.3 body lengths per second (bl s^{-1}), whereas trained fish experienced daily sessions of exercise for 6 h, 5 days a week, for a period of 4 wk, at a water flow rate of 0.9 bl s^{-1}. The patch-clamp technique was used to compare ion currents between groups. Trained fish exhibited higher whole cell capacitance, consistent with an increased membrane surface area of ventricular myocytes. Furthermore, exercise training led to reduced current densities of I_{Na}, I_{CaL}, and outward I_{K1}. These changes in currents were connected to marked alterations in AP morphology, including depolarized resting membrane potential (RMP), depolarized threshold potential (TP), and prolonged AP at 90% repolarization (APD_{90}). In summary, this study presents novel evidence that swimming exercise training can impact the ventricular ion currents, which leads to prolongation of the action potential, and that the cardiomyocytes of the rainbow trout are highly plastic, enabling them to respond to changes in the environment.

NEW & NOTEWORTHY Exercise training modulates cardiac ion currents, enlarges cardiomyocytes, and prolongs action potentials, demonstrating notable electrophysiological adaptations linked to enhanced cardiac performance in rainbow trout.

cardiomyocyte; electric excitation; exercise training; ion currents; rainbow trout

INTRODUCTION

Exercise training provides numerous health benefits across species, enhancing cardiac efficiency and preventing cardiovascular diseases in humans (1–4), rodents (5–8), mice (9), and swine (10). In fish, exercise training improves swimming performance, cardiovascular function, and overall physiological processes, contributing to enhanced fish welfare (11, 12). In rainbow trout (*Oncorhynchus mykiss*), exercise training has been shown to improve maximum cardiac performance, evidenced by higher stroke volume, cardiac output, maximum power output (13, 14), and maximum heart rate (15). However, the cellular mechanism underlying these adaptations remain poorly understood.

The heart, as the central pump of the circulatory system, distributes oxygen through coordinated contractions and relaxations initiated by electrical excitation of the sarcolemma in cardiac myocytes—a process known as the cardiac action potential (AP) (16, 17). The AP triggers myocyte contraction, with its shape influencing the regulation of cardiac excitation and contraction. Depolarization and repolarization phases of the AP are activated by the inward and outward currents of specific ion channels (18). Ion channels are membrane proteins that regulate the transport of ions across cell membranes and contribute to the electrical signals required to produce uniform contraction in the heart (18, 19). The main ions involved with AP are sodium (Na^+), calcium (Ca^{2+}), and potassium (K^+) (20).

In fish, as in other vertebrates, the cardiac AP can be divided to five phases (0–4) (21–23). During phase 4, atrial and ventricular cardiomyocytes maintain a stable resting membrane potential (RMP) of approximately −70 to −90 mV, primarily due to outward K^+ movement through inward rectifier potassium channels (I_{K1}). Depolarization (phase 0) is initiated by voltage changes from adjacent myocytes, triggering Na^+ influx through sodium channels. Early repolarization

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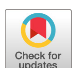

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(phase 1) involves K^+ outflow current. However, this phase is often less pronounced in fish compared with mammals (23–25). The plateau (phase 2) results from a balance between the inward Ca^{2+} and outward K^+ currents, maintaining a stable membrane voltage. Final repolarization (phase 3) is driven by outward K^+ currents, particularly I_{K1} and delayed rectifier potassium current (I_{Kr}), restoring the RMP and enabling diastolic filling (23, 26). In fish cardiac myocytes, I_{K1} stabilizes the RMP and aids late repolarization, whereas I_{Kr} facilitates AP repolarization and regulates heart rate variability (23, 27).

To the best of our knowledge, no studies have investigated how exercise training affects ion currents and action potentials in fish, despite their critical role in regulating cardiac excitability and contractility (20, 23). The present study aims to address this gap by examining the effects of exercise training on sodium (I_{Na}), L-type calcium (I_{CaL}), and potassium (I_{K1} and I_{Kr}) currents, as well as action potential (AP) characteristics, in ventricular myocytes of rainbow trout (*O. mykiss*). These findings will improve our understanding of cellular mechanisms underlying exercise-induced cardiac adaptations and their implications for cardiac plasticity in ectothermic vertebrates.

MATERIALS AND METHODS

Fish

Eighty-four healthy rainbow trout (*O. mykiss*) were randomly divided into two groups: untrained (control) and trained fish. The average weight was 1.51 ± 0.06 kg for control group and 1.48 ± 0.07 kg for trained group, whereas the average lengths were 44.7 ± 0.87 cm and 44.0 ± 0.57 cm for control and trained group, respectively. The fish were raised in aquaculture facilities of Natural Resources Institute Finland (Luke) located in Enonkoski. All-female rainbow trout stocks were used, produced via standard aquaculture sex-reversal feminization procedure at Luke. The experiment was conducted with 14 fish in each 1 m × 95 cm (height × diameter) tank, with three replicate tanks per group. Control fish were reared under standard aquaculture conditions, whereas trained fish experienced daily training sessions lasting 6 h, 5 days a week. The training procedure is described in *Training Protocol*. During the experimental period, an automated feeding system supplied the fish with commercial floating pellets (Ova 7S, Alltech Fennoaqua, Raisio, Finland) starting 1 h after training ended (03:30 PM) until sunset (10:00 PM). Environmental conditions, such as temperature, pH, and oxygen levels, remained relative stable within natural variations, with averages of 18.8 ± 2.1°C, 8.0 ± 0.2, and 6.9 ± 0.4 mg L^{-1}, respectively. The experiment was conducted from early August to the end of September 2023, and all procedures were approved by the National Project Authorisation Board (permission ESAVI-25297-2023).

Training Protocol

Before the training period, the fish were anesthetized with 100 ppm buffered tricaine methanesulfonate (MS-222) (Finquel, Argent Chemical Laboratories, Redmond, WA) to record their body weight and length. Water supply to tanks was adjusted with valves to control the water flow velocity. The freshwater was coming to hatchery from a nearby lake Ylä-Enonvesi. To maintain a consistent water flow velocity, the water input valves were adjusted to keep the velocity in the control tanks constant at 0.11 m s^{-1} [equivalent to 0.3 body lengths per second (bl s^{-1})], which is the regular velocity used in hatchery for this size rainbow trout. In the training tanks, two additional pumps were installed, increasing the water flow rate to 0.39 m s^{-1} (equivalent to 0.9 bl s^{-1}). The training intensity was selected based on the previous study, which has shown beneficial cardiac effects with velocity of 1 bl s^{-1} (28). The fish were trained for 6 h per day, from 08:30 AM to 02:30 PM, five consecutive days each week, for a period of 4 wk. After each training session, the fish were allowed to recover to prevent excessive stress or overtraining. Weekends also served as rest period. An automatic timer was used to turn off the pumps during rest periods, and during these times, the water flow velocity in the training tanks returned to the control speed. Water flow velocities in the experimental tanks were monitored three times weekly at the mid-water level of three different areas where the fish frequently swim, using a velocity meter (MiniAir20 + MiniController MC20, Schiltknecht Messtechnik, Gossau, Switzerland).

Myocyte Isolation

The ventricular myocyte isolation procedure followed the method outlined specifically for fish (29). After the exercise training, to initiate the process, the fish was stunned by a sharp blow to the head and then euthanized by severing the spine. Subsequently, the heart was promptly removed and placed in a perfusion system, where it was retrogradely perfused with nominally Ca^{2+} -free, low-Na^+ solution (Table 1) within a 10-min time frame from a height of 50 cm using hydrostatic pressure. The heart tissue was then subjected to enzymatic perfusion using a fresh low-Na^+ solution of collagenase (Type IA; 0.75 mg mL^{-1}, Sigma, St Louis, MO), trypsin (Type IX; 0.5 mg mL^{-1}, Sigma), and fatty acid-free bovine serum albumin (0.75 mg mL^{-1}, Sigma) for 20 min using a peristaltic pump. Continuous oxygenation with 100% O_2 was maintained in both perfusions to ensure supersaturation (>600 mmHg Po_2) and prevent hypoxia during enzymatic digestion, as per established protocols for cold-water teleost (29), with total exposure limited to <30 min to minimize potential oxidative stress. Following this, the softened ventricular tissue was digested with scissors in a small quantity of low-Na^+ solution, and individual myocytes were liberated by gently spinning muscle fragments using the aperture of a Pasteur pipette. The isolated myocytes were then stored at 4°C in a calcium-free low-Na^+ solution and used within 8 h of isolation. The total number of heart cells from trained and control fish used for the patch-clamp experiments were 107 cells and 66 cells, respectively.

Patch-Clamp Experiments

Enzymatically isolated ventricular myocytes were used to measure both ion currents and action potentials in the heart of the fish with previously standardized methods (26, 29, 30). A 150-µL recording chamber (RC-26, Warner Instruments, Hamden, CT) was filled with a small volume of ventricular myocyte suspension. The cells were allowed to settle and attach to a microscope slide at the bottom of chamber before

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Table 1. *The composition of internal (in; electrode) and external (out; bath) saline solutions used in current (I_{Na}, I_{CaL}, I_{Kr}, and I_{K1}) and action potential recordings*

	Experimental Solutions, mM									
	I_{Na}		I_{CaL}		I_{Kr}		I_{K1}		AP	
Compound	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**
NaCl	5	20		150		150		150		150
KCl					140	3	140	3	140	3
CsCl	130	120	20	130						
$CaCl_2$		0.5	2	1.8		1.8		1.8		1.8
$MgCl_2$	1	1	1	1	1	1.2	1	1.2	1	1.2
Mg_2ATP	5			5	4		4		4	
Na_2GTP									0.01	
EGTA	5			5	5		5			
HEPES	5	10	10	10	10	10	10	10	10	10
Glucose		10	10			10		10		10
TTX				0.0005		0.0005		0.0005		
Nifedipine		0.01				0.01		0.001		
E-4031								0.004		
$BaCl_2$						0.2				
TEA-Cl			120	15						
Oxaloacetate				5						
cAMP			0.02							
pH	7.2	7.7	7.7	7.2	7.2	7.6	7.2	7.6	7.2	7.6
Base	CsOH	CsOH	CsOH	CsOH	KOH	NaOH	KOH	NaOH	KOH	NaOH

Separate columns distinguish inward and outward components. AP, action potential; cAMP, cyclic adenosine monophosphate; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TEA-Cl, tetraethylammonium chloride; TTX, tetrodotoxin.

being externally superfused under hydrostatic pressure at a rate of 1.5–2.0 mL min^{-1} with a specially prepared saline solution for each measured ionic currents and action potential (Table 1). The temperature of the external solution was maintained at 12°C using a Peltier device (TC-10, Dagan, Michigan). Sharp-tipped patch electrodes were prepared from borosilicate glass capillary (King Precision) using a two-stage vertical electrode puller (Narishige PP-83, Japan), filled with a specific pipette solution for each ionic current and action potential (Table 1), and connected to the amplifier via a chlorinated silver wire electrode. Electrodes with resistance of 1.8 ± 0.1 MΩ (control) and 2.1 ± 0.2 MΩ (trained) were positioned on the cell membrane using a micromanipulator (Narishige MMO-23, Japan). Electrode capacitances (control: 8.60 ± 0.2 pF; trained: 8.83 ± 0.1 pF) were compensated after the formation of gigaohm seal. The membrane was ruptured by a brief voltage pulse (zap), and capacitive transients were eliminated by compensating for series resistance (control: 5.7 ± 0.6 MΩ; trained: 6.19 ± 0.8 MΩ) and cell capacitance (control: 62.6 ± 5.4 pF; trained: 67.1 ± 5.9 pF). Electrophysiological recordings were performed using an Axopatch 1-D amplifier with a CV-4 1/100 headstage (Axon Instruments, California), with data acquisition via Clampex 9.2 software and offline analysis using Clampfit 10.7 software (Axon Instruments). Recordings were conducted in either the voltage-clamp mode or the current-clamp mode, depending on whether ion currents or action potentials were recorded, respectively.

Sodium Current (I_{Na}) Experiments

I_{Na} measurements were conducted by eliciting currents from a holding potential (HP) of −120 mV at 2-s intervals. Recordings were performed within the voltage range of −100 to +70 mV for a duration of 60 ms, with 10 mV increments. About 80% of the series resistance was compensated to ensure precise control of the membrane voltage during the activation of fast and large I_{Na} currents (31). The currents were recorded at a sampling rate of 10 kHz and subsequently low-pass filtered at 5 kHz. The compositions of the external and internal (electrode) saline solutions used for I_{Na} measurement are presented in Table 1. To prevent I_{CaL} currents, nifedipine (Nif; 10 μmol L^{-1}; Sigma) was added to the external solution.

Voltage dependence of steady-state activation was determined from the current-voltage (*I-V*) relationships. Peak I_{Na} at each test potential was converted to conductance (*G*) using the equation:

$$G = \frac{I_{Na}}{V - V_{rev}},$$

where V is the test potential, and V_{rev} is the Na^+ reversal potential. The resulting conductance values were normalized to the maximum conductance (G/G_{max}) and fitted to a Boltzmann function:

$$G/G_{max} = \frac{1}{1 + \exp((V_{0.5} - V)/k)},$$

where $V_{0.5}$ is the half-activation voltage, and k is the slope factor. Steady-state inactivation was assessed using conditioning pulses from −120 to −20 mV in 10 mV increments, followed by a 20-ms test pulse to −30 mV to elicit I_{Na}. Peak current during the test pulse was expressed as a fraction of maximum current and fitted to a Boltzmann function:

$$\text{Availability} = \frac{1}{1 + \exp((V - V_{0.5})/k)},$$

where $V_{0.5}$ is the half-inactivation voltage, and k is the slope factor.

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Calcium Current (I_{CaL}) Experiments

I_{CaL} was induced from a −80 mV holding potential (HP) using 300-ms depolarizing pulses with 10 mV increments every 10 s within the voltage range of −80 to +30 mV. Myocytes with stable I_{CaL} after 3–5 min of internal perfusion were accepted for the experiments. I_{CaL} was calculated as the difference between the peak inward current and the current at the end of the 300-ms pulse. The compositions of the external and internal (electrode) saline solutions for I_{CaL} measurement are presented in Table 1. Cs^+-based and K^+-free solutions were used on both sides of the cell membrane to minimize interference with K^+ currents (29). Tetrodotoxin (TTX; 0.5 μmol L^{-1}; Sigma) was added to the external solution to completely block voltage-gated I_{Na} currents. I_{CaL} inactivation kinetics were assessed from voltage steps ranging from −80 to +30 in 10 mV increments from holding potential of −80 mV. For each test potential, the decay of I_{CaL} during 300-ms depolarizing pulse was fitted with a biexponential function to obtain time constant τ (tau). The resulting time constants were plotted as a function of membrane potential to generate τ-V relationships, using both absolute values and values normalized to the maximal time constant. To quantify the voltage dependence of inactivation kinetics, the τ-V data were fitted with a Boltzmann equation:

$$\tau(V) = \tau_{min} + \frac{\tau_{max} - \tau_{min}}{1 + \exp((V - V_{0.5})/k)},$$

where $V_{0.5}$ is the half-inactivation voltage, k is the slope factor, and τ_{min} and τ_{max} represent the lower and upper asymptotes of the voltage-dependent time constant, respectively. This approach provided estimates of the voltage dependence of I_{CaL} inactivation for myocytes from both control and trained fish.

Inward Rectifier Potassium Current (I_{K1}) Experiments

I_{K1} was assessed by applying a 1-s repolarizing voltage ramp from +60 to −120 mV every 10 s, starting from a holding potential (HP) of −80 mV (26). The compositions of the external and internal (electrode) saline solution for I_{K1} measurements are presented in Table 1. TTX (0.5 μmol L^{-1}), Nif (10 μmol L^{-1}), and E-4031 (4 μM) were added to the external solution to completely block voltage-gated I_{Na}, I_{CaL}, and I_{Kr} currents, respectively. I_{K1} components, both inward and outward, were determined by subtracting the current in the presence of Ba^{2+} (0.2 mmol L^{-1}) from the initial recording.

Delayed Rectifier Potassium Current (I_{Kr}) Experiments

I_{Kr} was evaluated through a two-step protocol (26). Initially, channels were activated to varying degrees by subjecting them to a series of depolarizing prepulses (lasting 4 s each) from a holding potential (HP) of −80 to +80 mV in 20 mV increments, followed by a subsequent voltage step to −40 mV for 4 s to remove inactivation. I_{Kr} tail current was then determined at −20 mV, maintaining a constant electrochemical driving force. The compositions of the external and internal (electrode) saline solutions for I_{Kr} measurements are presented in Table 1. TTX (0.5 μmol L^{-1}), Nif (10 μmol L^{-1}), and Ba^{2+} (0.2 mmol L^{-1}) were added to the external solution to completely block voltage-gated I_{Na}, I_{CaL}, and I_{K1} currents, respectively.

Action Potential Experiments

Ventricular myocytes were stimulated with current pulses of constant duration (4 ms) and increasing amplitude. The initial stimulus intensity was 300 pA, which was then increased in 20 pA increments until an all-or-none action potential (AP) was elicited. The stimulation frequency was set at 1 Hz, with one pulse delivered per second. The aim of this approach was to determine the threshold at which the myocytes would respond with a full AP, providing insight into their excitability and electrical properties. The following AP parameters were analyzed: resting membrane potential (RMP; mV), threshold potential (TH; mV), critical depolarization (CD = TH − RMP; mV), overshoot (OS; mV), amplitude (AMP; mV), duration at 10%, 50%, and 90% repolarization level (APD_{10}, APD_{50}, and APD_{90}; ms), the maximum rate of upstroke (+dV/dt; V/s), and the maximum rate of repolarization (−dV/dt; V/s) (Fig. 4*A*). The compositions of the external and internal solutions for AP measurement were presented in Table 1.

Data Statistical Analysis

All data are reported as means ± SE. After assessing the normality of the distribution, two-way ANOVA with Tukey's post hoc tests were used to compare current densities between groups in each holding potential. An unpaired Student's t test was performed to evaluate statistically significant differences in the size of the fish and in whole cell capacitance (C_m) as a proxy for myocyte size, as well as in AP variables and maximum current density values between the control and training groups. Linear regression analyses were performed between C_m and absolute and normalize ion currents (I_{Na}, I_{CaL}) for each cell, and regression lines were fitted using the least-squares method. Associations were reported as R^2 values. These analyses were used to evaluate whether observed changes in current densities could be attributed to cell size, supporting interpretation of genuine ion channel remodeling. Statistical analyses were conducted using SigmaPlot 15 software (Systat Software Inc., San Jose, CA), and a P value of < 0.05 was considered statistical significance.

RESULTS

Body Weight and Length of Fish

There were no differences in body weight or length between the control and trained groups, both before and after the 4-wk exercise training period ($P > 0.05$). After the training period, the control group consisted of 14 fish with an average weight of 1.88 ± 0.3 kg and an average length of 50.7 ± 0.9 cm, whereas the trained group included 16 fish with an average weight of 1.76 ± 0.1 kg and an average length of 49.4 ± 0.8 cm, which were used for the patch-clamp experiment.

Capacitance of Ventricular Myocytes

Ventricular myocytes from the trained group (65.3 ± 3.3 pF) had significantly greater capacitance compared with those in the control group (61.2 ± 2.9 pF) ($P < 0.05$).

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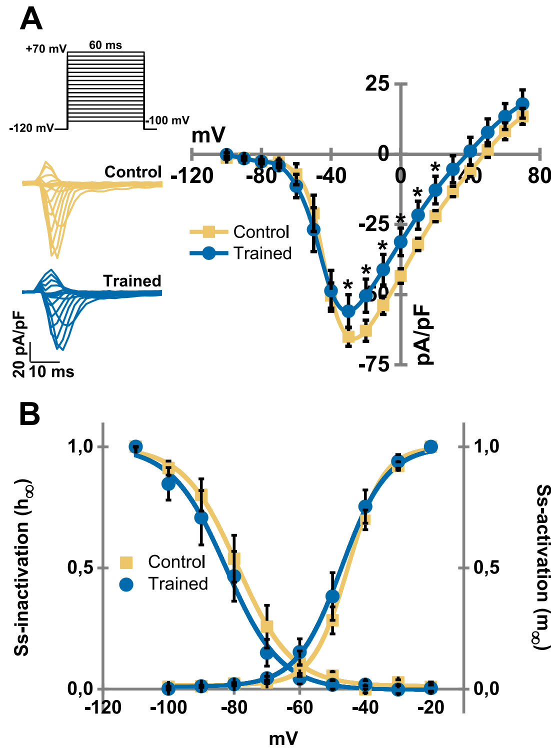

Figure 1. Effect of exercise training on Na^+ current (I_{Na}) in rainbow trout ventricular myocytes. Myocytes were isolated from 3–5 fish per experimental group. *A*: voltage protocol and representative current traces of I_{Na} in control and trained groups (*left*). Current-voltage (*I-V*) dependence of I_{Na} in control ($n = 15$ myocytes) and trained ($n = 13$ myocytes) groups (*right*). *B*: voltage dependence of steady-state (Ss) inactivation and activation of I_{Na} in control ($n = 10$ and 17 myocytes for Ss-inactivation and Ss-activation, respectively) and trained ($n = 7$ and 13 myocytes for Ss-inactivation and Ss-activation, respectively) groups. Asterisks indicate statistically significant differences between control and trained groups ($P < 0.05$, unpaired *t* test).

Sodium Current (I_{Na}) in Ventricular Myocytes

The current-voltage (*I-V*) relationship of sodium currents (I_{Na}) was measured by activating the channels with 60-ms voltage pulses ranging from −100 mV to +70 mV in 10 mV increments, with the peak current occurring at −30 mV. In trained fish, I_{Na} was considerably smaller between −30 and +20 mV compared with control fish ($P < 0.05$). Furthermore, at −30 mV, the maximum I_{Na} in the ventricular myocytes of control fish was significantly higher (−65 ± 0.6 pA/pF) as compared with myocytes from trained fish (−55.9 ± 1.3 pA/pF, $P < 0.05$) (Fig. 1*A*, Table 2). The voltage dependence of steady-state activation and inactivation of I_{Na} was similar between the control and trained groups (Fig. 1*B*, Table 2). The half-activation voltage ($V_{0.5}$, act) was −44.8 ± 1.3 mV in control and −47.0 ± 2.3 mV in trained myocytes, with comparable slope factors ($k = 4.7 \pm 0.2$ and 4.4 ± 0.3, respectively; $P > 0.05$). Likewise, the voltage dependence of steady-state inactivation showed no significant differences between groups (Fig. 1*B*, Table 2): the half-inactivation voltage ($V_{0.5}$, inact) was −78.9 ± 2.9 mV in control and −83.4 ± 3.7 mV in trained fish, and slope factors were similar ($k = -5.5 \pm 0.2$ and -5.3 ± 0.5; $P > 0.05$). These results indicate that exercise training did not alter the voltage sensitivity or gating kinetics of Na^+ channel activation and inactivation in ventricular myocytes.

Calcium Current (I_{CaL}) in Ventricular Myocytes

The L-type calcium current (I_{CaL}) was activated by repeated 60-ms depolarizing pulses across a voltage range from −80 mV to +30 mV, with 10 mV increments, peaking at 0 mV. From −10 mV to +30 mV, the I_{CaL} in trained fish was markedly reduced compared with untrained fish ($P < 0.05$) (Fig. 2*A*). The maximum I_{CaL} currents were −3.2 ± 0.4 and −5.0 ± 0.4 pA/pF in the ventricular myocytes of trained and control fish, respectively, at the voltage of 0 mV ($P < 0.05$) (Fig. 2*A*, Table 2). The half-inactivation voltage ($V_{0.5}$, inact) did not differ significantly between control and trained myocytes (19.6 ± 1.1 mV vs. 18.1 ± 1.0 mV, $P > 0.05$) (Fig. 2*B*, Table 2). However, the slope factor (k) was steeper in trained fish compared with controls (3.6 ± 0.5 vs. 2.1 ± 0.2, $P < 0.05$), indicating that the voltage sensitivity of channel inactivation was altered by exercise training. These results suggest that although the voltage at which half of the channels are inactivated remains unchanged, the rate of inactivation over voltage is modulated in trained ventricular myocytes (Fig. 2*B*, Table 2).

Table 2. *Peak current densities (pA/pF) and gating parameters for I_{Na} and I_{CaL} in ventricular myocytes from control and trained rainbow trout*

		Experimental Group			
	Peak Voltage, mV	Control	*n*	Trained	*n*
Sodium current (I_{Na}), pA/pF	−30	−65.0 ± 0.6*	15	−55.9 ± 1.3*	13
I_{Na} steady-state activation $V_{0.5}$, mV		−44.8 ± 1.3	17	−47.0 ± 2.3	13
I_{Na} steady-state activation slope, mV		4.7 ± 0.2	17	4.4 ± 0.3	13
I_{Na} steady-state inactivation $V_{0.5}$, mV		−78.9 ± 2.9	10	−83.4 ± 3.7	7
I_{Na} steady-state inactivation slope, mV		−5.5 ± 0.2	10	−5.3 ± 0.5	7
Calcium current (I_{CaL}), pA/pF	0	−5.0 ± 0.4*	16	−3.2 ± 0.4*	18
I_{CaL} steady-state inactivation $V_{0.5}$, mV		19.6 ± 1.1	14	18.1 ± 1.0	14
I_{CaL} steady-state inactivation slope, mV		2.1 ± 0.2*	14	3.6 ± 0.5*	14
Inward rectifier potassium current (I_{K1}, inward), pA/pF	−120	−11.6 ± 2.0	10	−8.6 ± 0.8	23
Inward rectifier potassium current (I_{K1}, outward), pA/pF	−60	2.3 ± 0.3*	10	1.6 ± 0.1*	23
Delayed rectifier potassium current (I_{Kr}), pA/pF	+40	1.2 ± 0.1	15	1.5 ± 0.2	30

Values are means ± SEM; n = number of cells (as indicated in the table) from 3–8 fish per group. Asterisks indicate a statistically significant difference ($P < 0.05$, unpaired t test) between control and trained groups. Peak currents were measured using step or ramp protocols (see MATERIALS AND METHODS). Gating parameters ($V_{0.5}$ = half-maximal voltage; slope = slope factor in mV) were derived from Boltzmann fits to steady-state activation/inactivation curves (see MATERIALS AND METHODS).

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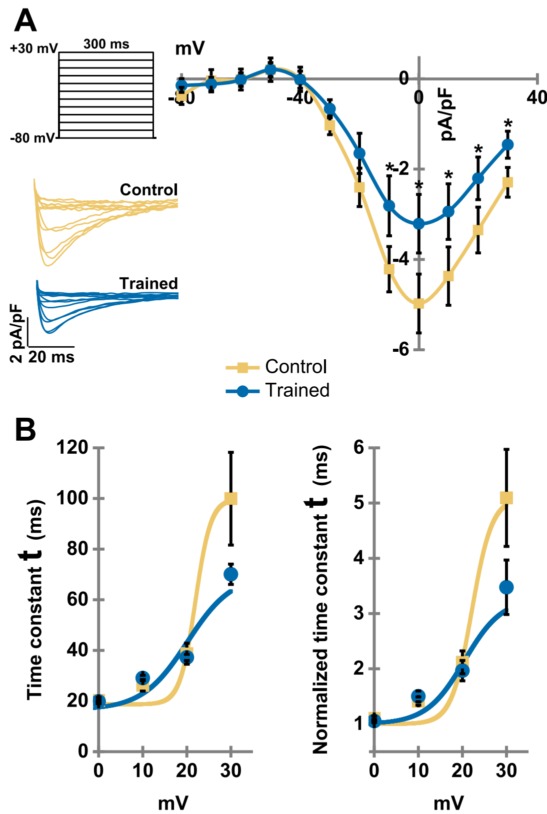

Figure 2. Effect of exercise training on Ca^{2+} current (I_{CaL}) in rainbow trout ventricular myocytes. Myocytes were isolated from 3–4 fish per experimental group. *A*: voltage protocol and representative current traces of I_{CaL} in control and trained groups (*left*). Current-voltage (*I-V*) dependence of I_{CaL} in control (n = 16 myocytes) and trained (n = 18 myocytes) groups (*right*). *B*: time constant of I_{CaL} measured in the same myocytes as in *A*. *Left*: absolute values of the time constants. *Right*: time constants normalized to the same peak amplitude (0 mV) to illustrate the effect of exercise training on current kinetics, including changes in V_{50} and slope value. Asterisks indicate statistically significant differences between control and trained groups ($P < 0.05$, unpaired *t* test).

Regression Analyses between Cell Capacitance and Sodium and L-Type Calcium Currents

Regression analyses were made to analyze whether the changes in current densities depended on the increase of cell size or via true channel remodeling. The results between cell capacitance (C_m) and both sodium (I_{Na}) and L-type calcium (I_{CaL}) currents indicated that the observed reductions in current densities in trained myocytes cannot be attributed solely to the increased cell size since I_{Na} (R^2 = 0.034, P = 0.55) and I_{CaL} (R^2 = 0.014, P = 0.65) densities showed little to no correlation with C_m in trained fish (Table 3).

Inward Rectifier K^+ Current (I_{K1}) in Ventricular Myocytes

The I_{K1} was elicited every 10 s by 1-s repolarizing voltage ramps from +60 mV to −120 mV and measured as a Ba^{2+}-sensitive current. Ba^{2+} was applied to completely block the inward rectifier K^+ channels, and subtraction of the current in the presence of Ba^{2+} from the pre-Ba^{2+} recording resulted in the typical I_{K1}, comprising both inward and outward current components. Exercise training did not affect the inward of I_{K1} in the trout myocytes ($P > 0.05$) (−11.6 ± 2.0 and −8.6 ± 0.8 pA/pF), but it did significantly impact the outward component of I_{K1} current (2.3 ± 0.3 and 1.6 ± 0.1 pA/pF), indicating that the I_{K1} outward current in control fish was much larger than in trained fish ($P < 0.05$) (Fig. 3*A*, Table 2).

Delayed Rectifier K^+ Current (I_{Kr}) in Ventricular Myocytes

The I_{Kr} current density was measured from the outward tail current peak generated by nine 4-s square-wave depolarizing pulses, ranging from −80 mV to +80 mV, each followed by a 4-s deactivating test pulse to −20 mV. As shown in Fig. 3*B* and Table 2, at +40 mV, the maximum I_{Kr} in the ventricular myocytes of control fish was 1.2 ± 0.1 pA/pF, whereas in trained fish, it was 1.5 ± 0.2 pA/pF. The difference between the two groups was not statistically significant ($P > 0.05$).

Action Potentials in Ventricular Myocytes

To investigate the cellular effects of exercise training, we measured the ventricular action potential (AP) responses to physical exercise in rainbow trout ventricular myocytes. Myocytes were stimulated with 4-ms current pulses of increasing amplitude in current-clamp mode at a pacing rate of 1.2 Hz and a temperature of 16°C to determine the minimum stimulus intensity required to induce an AP in the presence of an extracellular potassium concentration of 3 mmol L^{-1}. When the stimulus was sufficient to depolarize the membrane to the threshold potential (V_{TH}), an all-or-none AP was elicited, characterized by a rapid upstroke (Fig. 4, *A* and *B*). Exercise training led to modifications in several ventricular AP characteristics (Fig. 4*B*). The resting membrane potential (RMP) was significantly different

Table 3. *Linear regression analyses between cell capacitance (C_m) and sodium (I_{Na}) or L-type calcium (I_{CaL}) currents in control and trained ventricular myocytes*

Current	Analysis	Group	Equation	R^2	*P* Value
I_{Na}	pA vs. pF	Control	$Y = -42.52X - 1{,}802$	0.283	0.028
I_{Na}	pA vs. pF	Trained	$Y = -9.780X - 3{,}454$	0.034	0.5489
I_{Na}	pA/pF vs. pF	Control	$Y = 0.3046X - 91.74$	0.0695	0.307
I_{Na}	pA/pF vs. pF	Trained	$Y = 0.6202X - 105.1$	0.394	0.0216
I_{CaL}	pA vs. pF	Control	$Y = -2.745X - 116.3$	0.0612	0.3739
I_{CaL}	pA vs. pF	Trained	$Y = 0.4958X - 256.9$	0.0143	0.6471
I_{CaL}	pA/pF vs. pF	Control	$Y = 0.04123X - 7.199$	0.0477	0.434
I_{CaL}	pA/pF vs. pF	Trained	$Y = 0.06523X - 8.087$	0.4628	0.0027

Equations, determination coefficients (R^2), and P values are shown for each regression model. Sample sizes: I_{Na}: Control: n = 15 myocytes; Trained: n = 13 myocytes. Myocytes were isolated from 3–5 fish per group. Statistical analysis: simple linear regression using the least-squares method; P values indicate significance of the regression slope differing from zero.

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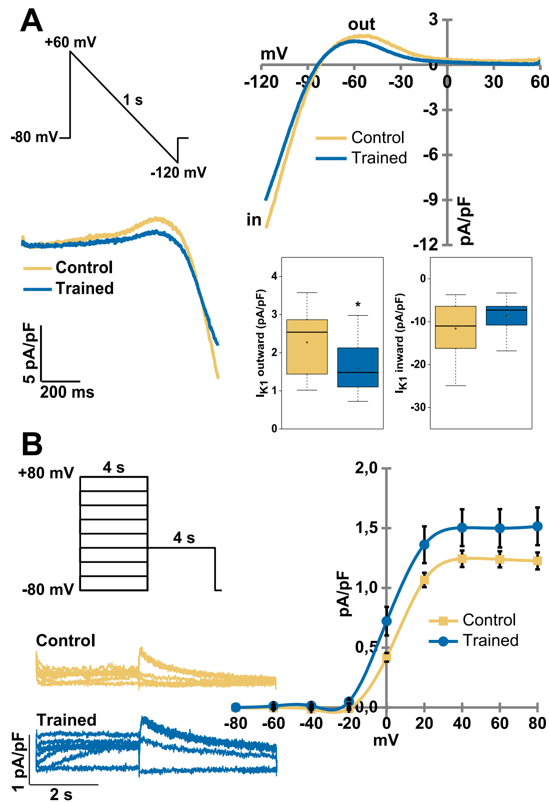

Figure 3. Effect of exercise training on K^+ current of rainbow trout ventricular myocytes. Myocytes were isolated from 4–8 fish per experimental group. *A*: voltage protocol and representative current traces of I_{K1} in control and trained groups (*left*). Current-voltage (*I-V*) dependence of outward and inward I_{K1} in control ($n = 10$ myocytes) and trained ($n = 23$ myocytes) groups (*right*). An asterisk indicates a statistically significant difference in outward I_{K1} between the control and trained groups ($P < 0.05$, unpaired *t* test). *B*: voltage protocol and representative current traces of I_{Kr} in control ($n = 15$ myocytes) and trained ($n = 30$ myocytes) groups. Current-voltage (*I-V*) dependence of I_{Kr} in control and trained groups (*left*) ($P > 0.05$, unpaired *t* test).

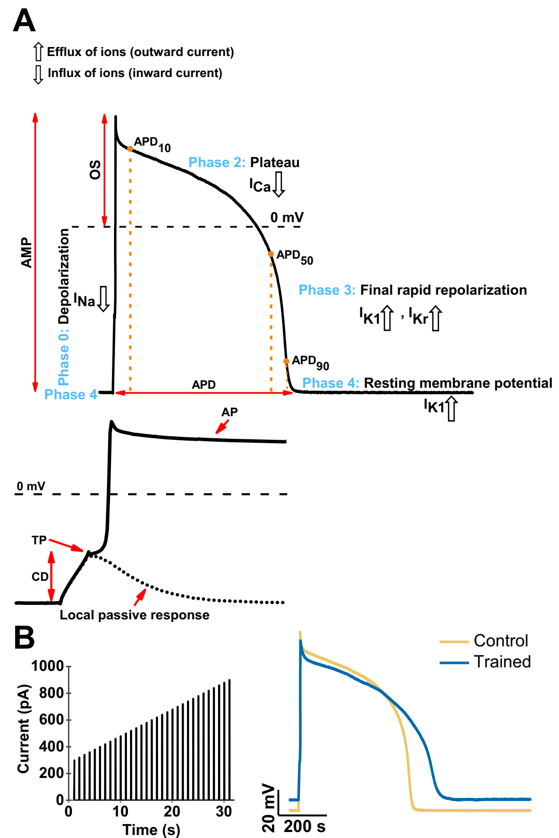

Figure 4. Effect of exercise training on action potentials (APs) of the rainbow trout ventricular myocytes. *A*: schematic representation of the ventricular AP, illustrating key parameters: resting membrane potential (RMP), AP amplitude (AMP), AP overshoot (OS), threshold potential (TP), critical depolarization (CD = TP − RMP), and AP duration at 10%, 50%, and 90% repolarization (APD_{10}, APD_{50}, and APD_{90}, respectively). *B*: voltage protocol and representative ventricular APs recorded from control ($n = 16$ myocytes from 3 fish) and trained ($n = 23$ myocytes from 3 fish) groups.

between the groups: −86.2 ± 0.6 mV for control and −81.6 ± 0.6 mV for trained fish ($P < 0.05$). In addition, the action potential duration at 90% repolarization (APD_{90}) was significantly different between the groups: 526.6 ± 26.6 ms for control and 621.8 ± 21.0 ms for trained fish ($P < 0.05$). The threshold potential (TP) was also higher in the trained group (−46.3 ± 0.9 mV) compared with the control group (−49.0 ± 0.9 mV) ($P < 0.05$), as shown in Table 4. Furthermore, the action potential amplitude (AMP), overshoot (OS), critical depolarization (CD), and action potential duration at 10% repolarization (APD_{10}) were slightly reduced in the trained group, although the differences were not statistically significant. The APD_{50} was slightly lower in the control group, but the difference was not significant ($P > 0.05$) (Table 4).

DISCUSSION

The main aim of the current project was to find out whether exercise training induces changes in the ion currents and action potential of ventricular myocytes of rainbow trout. According to our results, the ion current densities are extremely plastic and responsive to training as 4 wk of exercise training induced a significant reduction in sodium and calcium current densities and potassium outward current densities. These changes were, on the one hand, connected to the significant prolongation of the action potential

Table 4. *Characteristics of ventricular action potentials in control and trained rainbow trout myocytes*

	Experimental Group			
	Control	*n*	Trained	*n*
RMP, mV	−86.2 ± 0.6*	16	−81.6 ± 0.6*	23
AMP, mV	133.6 ± 2.2	16	129.1 ± 1.5	23
OS, mV	47.5 ± 1.8	16	47.4 ± 1.5	23
TP, mV	−49.0 ± 0.9*	16	−46.3 ± 0.9*	23
CD, mV	37.1 ± 1.1	16	35.3 ± 0.8	23
APD_{10}, ms	121.7 ± 20.3	16	93.0 ± 11.4	23
APD_{50}, ms	477.4 ± 25.8	16	519.6 ± 21.4	23
APD_{90}, ms	526.6 ± 26.6*	16	621.8 ± 21.0*	23
+d*V*/d*t*, V/s	206.1 ± 14.3	16	190.8 ± 7.3	23
−d*V*/d*t*, V/s	−10.5 ± 1.5	16	−8.9 ± 1.3	23

Values are means ± SE; n = number of cells (as indicated in the table) from 3 fish per group. Asterisks indicate a statistically significant difference ($P < 0.05$; unpaired t test) between the control and trained groups. $+dV/dt$, maximum upstroke rate; AMP, amplitude; AP, action potential; APD10, APD50, and APD90, duration at 10%, 50%, and 90% repolarization level; CD, critical depolarization; $-dV/dt$, maximum repolarization rate; OS, overshoot; RMP, resting membrane potential; TP, threshold potential.

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duration of myocytes from trained fish. This is the first study, to the best of our knowledge, showing how training can induce the alterations to ion currents and, thus, action potentials on fish.

Exercise-Induced Increase in Ventricular Myocyte Capacitance

Cell membrane capacitance, measured during the live cell patch-clamp experiments, is proportional to the cell surface area, and commonly used to account for cell size-dependent variations in ion current measurements (32). Consistent with previous research in rats (33, 34), the current study demonstrated that exercise training increases whole cell capacitance in rainbow trout, indirectly indicating an increase in cell size. The enhancement in the size of myocytes may lead to increased contractile force because the sarcolemmal membrane is integral to excitation-contraction coupling (35). Exercise training has previously been shown to induce increases in ventricular cell size in several mammalian species (36), potentially contributing to enhanced maximum power output. Similarly, in rainbow trout, training has been reported to increase the maximal cardiac capacities such as maximum power output, heart rate, stroke volume, and cardiac output (13, 15, 28). However, increasing cell size may also increase diffusion distances for oxygen and ions, which could affect metabolic efficiency and fatigue resistance (37). Thus, in future studies, it should be investigated further whether the increase of cell size is benefiting the heart or not. Furthermore, in the future, more direct measurements of cell size should be done, for example, via images of isolated myocytes, since current results give merely an estimate, and our results should be treated carefully when interpreting the results.

Reduction of Sodium Current (I_{Na}) in Trained Ventricular Myocytes

The cardiac sodium current (I_{Na}) is the first current activated during cardiomyocyte excitation through rapid Na^+ influx via voltage-gated Na^+ channels, driving the action potential (AP) upstroke (phase 0) and enabling impulse propagation across the heart muscle (38–40). In the current study, exercise training significantly reduced I_{Na} density in rainbow trout ventricular myocytes, representing an important electrophysiological adaptation. Furthermore, steady-state activation and inactivation of I_{Na} revealed no significant differences in V_{50} or slope between control and trained myocytes, suggesting that sodium channel gating properties remain largely unaltered by exercise training. This indicates that the observed reduction in peak I_{Na} is unlikely to result from changes in channel kinetics, but rather from other mechanisms such as channel density or posttranslational modifications. The regression result between cell size and I_{Na} also supports this suggestion that channel density is truly reduced, and reduced density is not due to the increased size of the cells. However, in future studies, this should be confirmed with more direct molecular analyses.

The reduction in I_{Na} density probably slowed the action potential (AP) upstroke, as evidenced by a reduced maximum rate of depolarization ($+dV/dt$). This reduction in myocyte excitability likely contributes to the stabilization of cardiac rhythm by decreasing the propensity for premature excitations. This represents a novel finding in fish hearts and parallels antiarrhythmic adaptations observed in mammals (34, 41). The observed reduction in I_{Na} density may also influence calcium handling indirectly. By prolonging the early phase of the AP, it extends the time window for L-type Ca^{2+} channel activity, potentially enhancing contractility despite reduced Ca^{2+} current density (18). Such adaptations may enhance cardiac stability under physiological stress, such as exercise, by minimizing the risk of ectopic beats or arrhythmias—similar to the mechanism in mammals, where moderated I_{Na} protects against reentrant arrhythmias (41). The reduction in I_{Na} density in trout may represent an adaptation to balance excitability and energy efficiency in variable aquatic environments, distinct from mammals, due to differences in Na^+ channel isoforms (40, 42, 43). Thus, future studies should investigate in more detail specifically how expression patterns of sodium channels change in response to training.

Decreased L-Type Calcium Current (I_{CaL}) in Response to Exercise Training

The L-type calcium channel (I_{CaL}) regulates voltage-dependent Ca^{2+} influx into cardiac myocytes, playing a central role in maintaining the plateau phase of the action potential and triggering excitation-contraction coupling (18, 44). Intracellular Ca^{2+} also influences Na^+ and K^+ channel activity, impacting overall cardiac excitability and rhythm (45). These mechanisms are essential during exercise and posttraining adaptation, supporting increased contractile performance and cardiac output (46). For the first time, we demonstrated that 4 wk of exercise training induced a significant reduction in L-type Ca^{2+} current (I_{CaL}) density, a similar finding as reported in rat myocardium (34). As in sodium channels, this reduction was not through the increase of cell size but likely due to changes in channel expression, which warrant further molecular investigations.

Our analysis of I_{CaL} inactivation kinetics revealed no differences in the voltage of half-inactivation (V_{50}) between groups; however, the slope of the Boltzmann fits was steeper in trained fish, indicating a more gradual transition between available and inactivated channel states. This kinetic adaptation may fine-tune Ca^{2+} entry dynamics during repetitive depolarizations. Together with the reduced peak I_{CaL} density, this may represent a coordinated response to limit Ca^{2+} influx and prevent overload while maintaining efficient excitation-contraction coupling during endurance-type activity. These observed changes in both density and inactivation could also contribute to the observed prolongation of AP in trained fish, especially phase 2 and the 90% repolarization phase (APD_{90}).

Although I_{NCX} was not directly measured, the preserved Ca^{2+} balance despite reduced I_{CaL} suggests that overall Ca^{2+} handling remained functionally compensated under the present experimental conditions. I_{CaL} responses to β-adrenergic stimulation were not directly assessed in this study either. Future experiments examining I_{CaL} modulation by isoproterenol would help clarify how β-adrenergic regulation interacts with exercise-induced cardiac remodeling in rainbow trout. Further immunostaining or dihydropyridine-binding studies could also verify sarcolemmal CaV1.2

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changes and complement the observed kinetics shifts in I_{CaL}, providing additional mechanistic insight into exercise-induced remodeling in fish cardiomyocytes.

Modulation of Potassium Currents (I_{K1} and I_{Kr}) by Exercise Training

Potassium (K^+) channels play a crucial role in the different depolarization phases of the cardiac action potential (AP), with distinct currents exerting specific effects on cardiac function (47). The inwardly rectifying potassium current (I_{K1}) contributes to different phases of the ventricular AP, including the stabilization of the resting membrane potential (RMP), initial depolarization, and final repolarization. Through these roles, I_{K1} critically influences cardiac excitability and susceptibility to arrhythmias, and also plays a key role in determining the success or failure of AP initiation (48, 49). In addition, the delayed rectifier potassium current (I_{Kr}) is essential for the rapid repolarization of ventricular myocytes (50).

Our results suggest that exercise training significantly reduced the outward component of I_{K1}, whereas the inward component remained unchanged. The decrease in outward I_{K1} probably contributes to the depolarized RMP in trained fish. This depolarization would conceivably amplify the responsiveness to sympathetic drive, potentially helping in maintaining higher cardiovascular demands during swimming (51); however, direct evidence in fish showing exercise-induced increases in adrenergic sensitivity is limited and requires future studies. The unchanged inward I_{K1} component suggests selective modulation of I_{K1} channel subtypes or regulatory mechanisms, potentially preserving the ability to maintain hyperpolarization during diastole.

The nonsignificant increase in I_{Kr} in trained fish suggests a modest enhancement of repolarization capacity, which may support faster recovery between contractions, potentially during sustained exercise. However, its limited impact compared with reductions in I_{K1} and I_{CaL} suggests that I_{Kr} contributes to AP shaping primarily through its interaction with I_{CaL} during the plateau phase (52). In mammals, exercise has been shown to enhance K^+ channel function, such as increased K_{ATP} channel expression in mice, leading to faster AP shortening with heart rate acceleration (53), and improved coronary artery function in miniature swine (54). In fish, the plasticity of K^+ currents in response to exercise training highlights their role in adapting cardiac function to environmental and physiological demands. Future studies should investigate the molecular mechanisms (e.g., Kir2, hERG expression) and assess their impact on whole heart electrophysiology using techniques like electrocardiography (ECG). In general, the current results, nevertheless, suggest that all ion channels modify their action in response to exercise training, which leads to significant changes in action potential.

Action Potential Prolongation and Ionic Contributions in Trained Myocytes

Following exercise training, ion currents in the ventricular cells of trained rainbow trout contributed to significant changes in the shape of AP, with notable differences observed in the RMP, TP, and APD_{90}. RMP was significantly depolarized in trained fish, which was probably due to a decrease in the outward component of the I_{K1}. This depolarization of RMP, as a previous report (26), reduces the stability of the resting phase, potentially increasing myocyte excitability and responsiveness to sympathetic stimulation (20), which is advantageous during exercise to meet the elevated cardiovascular demands. However, depolarized RMP may also increase the risk of ectopic depolarizations, potentially predisposing the heart to arrhythmias under stress, as noted in mammalian studies (48). The threshold potential (TP) was also significantly depolarized in trained fish, consistent with the reduced I_{Na} density, suggesting a slower AP upstroke and reduced excitability. Though this may stabilize rhythm during prolonged swimming (20, 41), simultaneous depolarization of RMP and TP may reduce the safety margin against arrhythmia under the influence of environmental stressors, especially during hypoxia or temperature stress (43).

Since the shape of the AP varies regionally in the heart (20), training-induced AP changes might affect multiple functional regions and contribute to overall cardiac performance. The observed shift in TP suggests increased excitability in trained cardiomyocytes, potentially facilitating earlier action potential generation (49). These changes may contribute to enhanced ventricular excitability, potentially supporting overall cardiac adaptations observed in trained individuals, such as improved performance metrics (13, 15). Meanwhile, primary heart rate adaptations likely occur via sinoatrial (SA) node hyperpolarization-activated cyclic nucleotide-gated (HCN) channel downregulation (55), completing these ventricular changes.

The most significant change in our study was a marked prolongation of APD_{90} in trained fish and, at the same time, a clear shortening of APD_{10}. This counterintuitive effect is due to a lower early plateau voltage that activates less I_{Kr}, since I_{Kr} activation is voltage-dependent and stronger at more depolarized membrane potentials. Thus, the prolongation of APD_{90} may not require a direct reduction in I_{Kr}, but may result indirectly from reduced I_{CaL} amplitude and its effects on plateau voltage and K^+ current activation (52). Despite the reduction in I_{CaL} density, the prolonged plateau phase (phase 2) likely allows continued calcium influx (18). This may help explain the extended cardiac contraction observed in trained fish, as a longer plateau allows more time for calcium entry, enabling greater cross-bridge formation between actin and myosin. Such prolongation may therefore enhance contraction force in addition to modifying rhythm. It has been emphasized that cardiac APs in fish are highly plastic and adapt to environmental and physiological demands (23), and our results suggest that exercise training induces coordinated ion flow remodeling to optimize cardiac performance. Prolonged APD may also affect resting heart rate, which is a common form of training adaptation in mammals (56), by prolonging the refractory period and optimizing contractile cycles, which reduces the number of beats required for effective cardiac output. However, this prolongation may increase the energy cost of contraction due to prolonged calcium handling, which may affect fatigue tolerance during prolonged exercise (22), warranting also future investigations.

Limitations

In current study, the cell size was inferred solely from capacitance, an indirect measure. Furthermore, ventricular or whole heart mass and direct morphometric data were not obtained due to methodological constraints, and calibrated

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microscopic morphometry was not feasible during patch-clamp recordings. In addition, I_{Na} tail currents and recovery kinetics could not be assessed, as the voltage-clamp recordings did not include dedicated prepulses or tail protocols. Future studies should combine capacitance recordings with imaging and heart mass measurements to confirm hypertrophy, and use dedicated voltage-clamp protocols to directly assess I_{Na} inactivation, recovery, and tail currents.

The limitation also includes that our measurements were done only with female rainbow trout. Since sex differences exist in trout cardiac electrophysiology (57), future mixed-sex studies could explore interactions. Furthermore, as temperature is having profound effect on ion channels in fish (22, 43), the differences between trained and untrained fish for thermal responses should be investigated at ion channel level since currently the investigations are merely at whole animal level (15, 28).

Conclusions

In conclusion, 4 wk of exercise training significantly altered the electrical excitability of ventricular myocytes in rainbow trout. Exercise training increased ventricular myocyte capacitance, indicating cellular hypertrophy, and reduced the densities of sodium (I_{Na}), L-type calcium (I_{CaL}), and the outward component of the inward rectifier potassium (I_{K1}) currents. These changes were associated with a prolonged action potential (APD_{90}), a depolarized RMP, and an elevated threshold potential (TP) in trained fish. These adaptations reflect the high plasticity of rainbow trout cardiomyocytes in response to exercise, with potential benefits for swimming performance. However, the depolarized RMP and reduced current densities may increase arrhythmia risk under environmental stressors, warranting further investigation. Future studies should explore the observed changes more throughout at the molecular level as well as investigate the whole heart electrophysiological changes using electrocardiography (ECG) and assess contractile force to fully elucidate the functional implications of these cellular adaptations in rainbow trout.

DATA AVAILABILITY

Data will be made available upon reasonable request.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.T.N.L., J.H., and K.A. conceived and designed research; T.T.N.L., E.S., J.H., and K.A. performed experiments; T.T.N.L. and J.H. analyzed data; T.T.N.L., J.H., and K.A. interpreted results of experiments; T.T.N.L. and J.H. prepared figures; T.T.N.L. drafted manuscript; T.T.N.L., E.S., J.H., and K.A. edited and revised manuscript; T.T.N.L., E.S., J.H., and K.A. approved final version of manuscript.

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