

8-1-2017

Ventricular Action Potential Adaptation to Regular Exercise: Role of β -adrenergic and K_{ATP} Channel Function

Xinrui Wang
Marquette University

Robert H. Fitts
Marquette University, robert.fitts@marquette.edu

Marquette University

e-Publications@Marquette

Biology Faculty Research and Publications/College of Arts and Sciences

This paper is NOT THE PUBLISHED VERSION; but the author's final, peer-reviewed manuscript. The published version may be accessed by following the link in the citation below.

Journal of Applied Physiology, Vol. 123, No. 2 (August 2017): 285-296. [DOI](#). This article is © American Physiological Society and permission has been granted for this version to appear in [e-Publications@Marquette](#). American Physiological Society does not grant permission for this article to be further copied/distributed or hosted elsewhere without the express permission from American Physiological Society.

Ventricular Action Potential Adaptation to Regular Exercise: Role of β -Adrenergic and K_{ATP} Channel Function

Xinrui Wang

Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin

Robert H. Fitts

Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin

Abstract

Regular exercise training is known to affect the action potential duration (APD) and improve heart function, but involvement of β -adrenergic receptor (β -AR) subtypes and/or the ATP-sensitive K^+ (K_{ATP}) channel is unknown. To address this, female and male Sprague-Dawley rats were randomly assigned to voluntary wheel-running or control groups; they were anesthetized after 6–8 wk of training, and myocytes were isolated. Exercise training significantly increased APD of apex and base myocytes at 1 Hz and decreased APD at 10 Hz. Ca^{2+} transient durations reflected the changes in APD, while Ca^{2+} transient amplitudes were unaffected by wheel running. The nonselective β -AR agonist isoproterenol shortened the myocyte APD, an effect reduced by wheel running. The isoproterenol-induced shortening of APD was largely reversed by the selective β_1 -AR blocker atenolol, but not the β_2 -AR blocker ICI 118,551, providing evidence that wheel running reduced the sensitivity of the β_1 -AR. At 10

Hz, the K_{ATP} channel inhibitor glibenclamide prolonged the myocyte APD more in exercise-trained than control rats, implicating a role for this channel in the exercise-induced APD shortening at 10 Hz. A novel finding of this work was the dual importance of altered β_1 -AR responsiveness and K_{ATP} channel function in the training-induced regulation of APD. Of physiological importance to the beating heart, the reduced response to adrenergic agonists would enhance cardiac contractility at resting rates, where sympathetic drive is low, by prolonging APD and Ca^{2+} influx; during exercise, an increase in K_{ATP} channel activity would shorten APD and, thus, protect the heart against Ca^{2+} overload or inadequate filling.

NEW & NOTEWORTHY Our data demonstrated that regular exercise prolonged the action potential and Ca^{2+} transient durations in myocytes isolated from apex and base regions at 1-Hz and shortened both at 10-Hz stimulation. Novel findings were that wheel running shifted the β -adrenergic receptor agonist dose-response curve rightward compared with controls by reducing β_1 -adrenergic receptor responsiveness and that, at the high activation rate, myocytes from trained animals showed higher K_{ATP} channel function.

heart disease is one of the leading causes of morbidity and mortality in the Western world, and programs of regular exercise training have been shown to ameliorate cardiovascular risk factors, reduce the incidence and severity of ischemic heart disease, and protect against heart failure (18, 36). Although the benefits of exercise in promoting health are well recognized, our knowledge regarding the cellular/molecular mechanisms of exercise-training-induced adaptations in the heart remains incomplete. One factor of importance is the action potential (AP) duration (APD) and the resulting Ca^{2+} transient, which are known to be regulated by sympathetic (adrenergic) activation (35, 44). Regulation of the cardiac APD during rest and exercise is important to ensure adequate Ca^{2+} influx and prevent Ca^{2+} overload, afterdepolarizations, excess ionic pump activity [Na^+ - K^+ and sarcoplasmic reticulum (SR) Ca^{2+} pumps], and/or inadequate time for relaxation (57). Exercise-induced changes in heart structure/function are not uniform across the wall of the heart (35, 40). Stones et al. (40) and Natali et al. (35) reported that wheel running in rats prolonged the AP in beating hearts in epicardial, but not endocardial, myocytes. In addition, voluntary wheel running in rats produced a significant increase in cell volume (both cell length and width) in endocardial, but not epicardial, myocytes (34). However, little is known about whether male and female hearts adapt differently to exercise training (50). Investigation of sex-specific adaptations is important, as differences in clinical outcomes are known; for example, females experience a greater incidence of QT prolongation, a disease associated with arrhythmias, such as torsades des pointes, which can lead to sudden cardiac arrest (2, 28). Thus, understanding how regular exercise may alter the AP and protect against arrhythmias is important. To gain a better understanding of regional and sex-specific effects of regular exercise, we studied myocytes isolated from the base (which consists mostly of the subendocardial layer) and apex (which consists mostly of the subepicardial layer) regions of the left ventricle in male and female rats.

Regulation of the ATP-sensitive K^+ (K_{ATP}) channel seems particularly important in controlling APD at a high heart rate, as the K_{ATP} channel is activated by a reduced ATP-to-ADP ratio, when metabolic demand exceeds oxygen supply (24, 57). K_{ATP} channel-dependent K^+ efflux accelerates repolarization, shortening the cardiac AP, which limits Na^+ and Ca^{2+} entry into the cell (37). This reduces energy requirements for ion homeostasis and maintains a diastolic interval adequate for myocardial relaxation. While exercise training and as few as 5 days of running have been shown to increase expression of the K_{ATP} channel, the extent to which regular exercise training affects K_{ATP} channel function and cardiac excitability in males and females and in the epicardium vs. the endocardium remains to be established (7, 57).

In addition to the K_{ATP} channel, APD is critically regulated by adrenergic agonists (44). Adrenergic receptors (ARs) are members of the G protein-coupled receptor superfamily of membrane proteins that mediate the actions of circulating catecholamines. β -AR stimulation by the sympathetic nervous system is broadly involved in peripheral blood circulation, metabolic regulation, muscle contraction, and central neural activities (52). In the heart, acute β -AR stimulation serves as the most powerful means to regulate cardiac output in response to a

fight-or-flight situation, whereas chronic β -AR stimulation plays an important role in physiological and pathological cardiac remodeling (6). Sympathetic nervous system regulation of cardiac APD is primarily mediated by β -AR activation (12, 30, 56). Three β -AR subtypes have been identified in the human heart. β_1 -ARs play a predominant role in regulating Ca^{2+} influx and inotropic responses (56). Similarly, β_2 -ARs also have a functional role in cardiomyocyte contraction, but their activation may be important during stress and conditions that cause β_1 -AR downregulation, such as heart failure (14, 33). Contrary to β_1 - and β_2 -ARs, β_3 -AR signaling leads to activation of a nitric oxide synthase pathway and is not involved in AP regulation (47). The effect of exercise training on the response of APD to β -AR subtype stimulation has not been tested.

In the present study we used 1-Hz stimulation to investigate how regular exercise alters the APD and the Ca^{2+} transient in resting unstressed left ventricular myocytes and 10-Hz stimulation to assess these properties under conditions of metabolic stress likely experienced during heavy exercise. Pharmacological methods were used to assess mechanisms and, in particular, to determine the relative importance of β -AR subtypes and the K_{ATP} channel in mediating the response to exercise training. This study contributes to a better understanding of how exercise training alters the electrical and functional properties of the base and apex regions of the heart in both sexes and sets the stage for the development of optimal exercise paradigms that maximize the benefits of regular exercise.

METHODS

Animals and Exercise Paradigms

All experiments and the protocol for animal care and use were approved by the Marquette University Institutional Animal Care and Use Committee. Age-matched male and female young adult (6- to 8-wk-old) Sprague-Dawley rats remained sedentary (SED, $N = 53$) or participated in voluntary wheel exercise training (TRN, $N = 43$) for 6–8 wk using a moderate (loaded-wheel running) training paradigm. The wheel-running protocol was similar to that described by Stones et al. (40), except the wheel was loaded with a resistance equal to 15% of the animal's body weight. Individual running distances were recorded daily. Sedentary animals were housed as standard laboratory rats with no access to wheels.

Myocyte Isolation

Control and trained male and female rats were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg body wt ip); depth of anesthesia was documented by loss of the reflex response to toe pinch. Hearts were rapidly extracted and weighed, attached to a cannula, and perfused on a modified Langendorff apparatus at 37°C for 5 min (or until all blood was washed out) with *solution A* (24 mM NaHCO_3 , 5 mM glucose, and 150 U heparin/0.1 ml) in Joklik (minimum essential medium Eagle) solution bubbled with 95% O_2 -5% CO_2 , pH 7.23. The perfusate was switched to *solution B* [60 ml of *solution A* + 60 mg of BSA, 14 mg of collagenase type II (250 U/mg), and 12 mg of protease type XIV (5.4 U/mg)], and the heart was perfused for 14–21 min (time dependent on the size of the heart) (19). After digestion, the left ventricle was cut away, and base and apex regions were minced separately in *solution B*, as described by Natali et al. (34), and incubated for 5 min in a 37°C shaker bath. The cell suspension was filtered (297- μm mesh) and centrifuged at 1,000 rpm for 4 min. The resulting pellet was resuspended in 0.5 mM Ca^{2+} and 10 mM HEPES-buffered Tyrode solution, centrifuged, and again resuspended in 0.5 mM Ca^{2+} -containing Tyrode solution (40). The isolated myocytes were loaded with fluo 4-AM or fura 2-AM (1 μg /250 μl of cell suspension dissolved in 100% DMSO) to monitor intracellular Ca^{2+} . After 45 min, the loading solution was exchanged with 2 mM Ca^{2+} and 10 mM HEPES-buffered Tyrode solution containing 10 mM glucose.

AP Characteristics

Myocytes were transferred to a recording chamber mounted on the stage of an inverted microscope (model TE2000, Nikon), where they adhered to laminin-coated glass coverslips. Quiescent rod-shaped myocytes were

selected and impaled with a fine-tip microelectrode (50–70 M Ω) filled with 2.7 M KCl and 10 mM HEPES. To characterize the effects of exercise training on the AP, we activated myocytes with 1-Hz, 1.0-nA, 3-ms pulses for 60 s (AxoClamp-2A). The purpose of the low pulse rate was to elicit “resting” APs unaffected by high activity, where the K_{ATP} channel would be activated. The AP was recorded at room (23°C) and body (37°C) temperatures. The 23°C experiments were performed, because myocytes are stable at this temperature, allowing multiple activations, and because many previous studies were conducted at room temperature (1, 35). The time (ms) from the beginning of the AP until membrane potential returned to 10% of its peak value [APD at 90% repolarization (APD₉₀)] was evaluated for the last 10 APs of each 60-s recording and averaged (Clampex). To determine how activity alters the AP, myocytes were pulsed at 10 Hz for 60 s. This pulse rate was selected, as it mimics heart rates obtained during heavy exercise in the rat (51).

Ca²⁺ Transient

Ca²⁺ transients were studied using fluo 4 and fura 2-AM. Both were used, as the ratiometric dye fura 2 allows determination of the Ca²⁺ transient amplitude independent of cell volume, but its relatively high Ca²⁺ affinity can cause cytosolic buffering, which might lengthen the Ca²⁺ transient, whereas the relatively low Ca²⁺ affinity of fluo 4 reduces this problem (39). Fluo 4 was excited at 480 nm, and fluorescence emission (520 nm) was monitored using a CoolSNAP camera and NIS-Elements imaging and acquisition module. Fura 2 was excited at 340 and 380 nm, with wavelength switched using a Lambda DG 4 ultra-high-speed wavelength switcher, and fluorescence emission was measured at 510 nm with a Q-Imaging Retiga R3 camera; emission was sampled at an interval of 20 ms for each excitation wavelength, and the ratio was analyzed with MetaFluor software (version 7.8). Whole cell Ca²⁺ transients and APs were monitored simultaneously at the start of stimulation and at 10-s intervals throughout the 60-s stimulation period. The peak amplitude and duration (from the beginning until return to baseline) were measured from three different sites along the myocytes and averaged. Myocyte width was determined from the average of measurements at three different sites along the cell, and length was determined using NIS-Elements or MetaMorph software.

β -AR Study

To examine if any endogenous β -AR activation existed in isolated myocytes, APs were elicited after administration (>10 min) of the nonselective β -AR antagonist propranolol (10 μ M; EMD Millipore). In separate experiments, dose-response curves for the nonselective β -AR agonist isoproterenol (EMD Millipore) were implemented. A given individual myocyte was superfused with 2 mM Ca²⁺-containing Tyrode solution for >2 min at each isoproterenol concentration from 10⁻¹¹ to 10⁻⁶ M. Selective β_1/β_2 -AR subtype stimulation was obtained under steady-state conditions after 10 min of exposure to a maximal-response dose of isoproterenol (10⁻⁶ M) in the presence of the selective β_2 -AR blocker ICI 118,551 (0.1 μ M; Tocris Bioscience) or the selective β_1 -AR antagonist atenolol (0.1 μ M; Acros Organic-Fischer Scientific). All β -AR studies were performed at 23°C with a 1-Hz pulse rate. The low pulse rate was selected to study the effect of β -AR stimulation without activation of the K_{ATP} channel.

K_{ATP} Channel Study

To study the effect of exercise training on the K_{ATP} channel, APs were elicited at 10 Hz before and after administration of the K_{ATP} blocker glibenclamide (2 μ M; Sigma). Myocytes were stimulated at 10 Hz to generate high metabolic demand, reducing the ATP-to-ADP ratio and, thus, activating K_{ATP} channels. To separate the relative importance of β -AR from the K_{ATP} effect, a given myocyte was stimulated at 1 Hz for 1 min (1 Hz) and then at 10 Hz while superfused for >2 min, in the following order, with 2 mM Ca²⁺-containing Tyrode solution (10 Hz), a maximal-response dose of isoproterenol (1 μ M) in 2 mM Ca²⁺-containing Tyrode solution (10 Hz + Iso), and 1 μ M isoproterenol + 2 μ M glibenclamide in 2 mM Ca²⁺-containing Tyrode solution (10 Hz + Iso + Gli). All K_{ATP} channel studies were performed at 23°C. The difference between the APD before addition of isoproterenol

and the APD after addition of isoproterenol represents the β -AR contribution, while the difference between the APD before addition of glibenclamide and the APD after addition of glibenclamide reflects the contribution of the K_{ATP} channel. The difference between the APD obtained at 1 Hz and the APD obtained 10 Hz in the presence of isoproterenol for a given myocyte represents the total AP shortening, which involves both sympathetic nervous system regulation and sarcolemma channel responses. Thus the percentage of the β -AR and K_{ATP} channel contributions was calculated as follows: %Iso = $[(10 \text{ Hz} - (10 \text{ Hz} + \text{Iso})) / (1 \text{ Hz} - (10 \text{ Hz} + \text{Iso}))]$ and % K_{ATP} = $[(10 \text{ Hz} + \text{Iso} + \text{Gli}) - (10 \text{ Hz} + \text{Iso})] / [1 \text{ Hz} - (10 \text{ Hz} + \text{Iso})]$.

Statistics

N and *n* represent the number of animals and myocytes, respectively. Values are means \pm SE. Wheel-running and cardiac morphological parameters were analyzed using one-way ANOVA. APD₉₀ and Ca²⁺ transient data (including Δ and %changes) were randomly obtained from different groups (male/female, SED/TRN, apex/base, 1 Hz/10 Hz, 23°C/37°C, and β_1/β_2 -AR) and compared by two-factor nested ANOVA, which allows comparison using the total number of fibers (*n*), rather than the number of rats (*N*). Dose-response curves to isoproterenol were analyzed by nested two-way ANOVA for repeated measures. Tukey's tests were used for all post hoc comparisons. Differences were considered significant for *P* < 0.05. ANOVAs were performed with Minitab 17. Isoproterenol dose-response curves were constructed with nonlinear regression, and the fitted midpoints (logI_{C50}) were obtained in GraphPad Prism 6.

RESULTS

[Figure 1](#) demonstrates the ability of male and female rats to perform voluntary wheel running. Daily running distances increased over the first 3 wk of training from 3.3 ± 0.5 to 10.6 ± 0.4 km for the males (*P* < 0.05) and from 6.9 ± 1.2 to 20.8 ± 0.6 km for the females (*P* < 0.05), with significantly greater daily running distance in the females (*P* < 0.05). Male rats ran at a speed of 1.3 ± 0.06 km/h, which was significantly slower than the running speed of female rats, which averaged 1.8 ± 0.05 km/h (*P* < 0.05). The higher running speed of the females partly explained their greater average running distance.

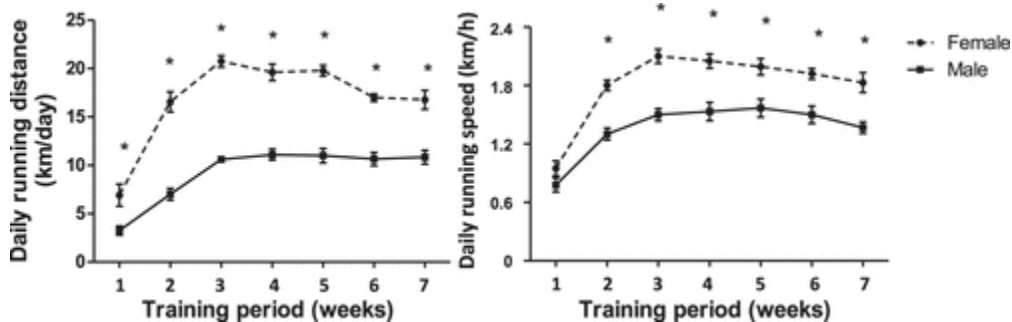


Fig. 1. Daily voluntary running distance and speed. Values are means \pm SE; *N* = 12 for both sexes. **P* < 0.05, male vs. female.

Voluntary exercise caused cardiac hypertrophy, as shown by an increased heart weight-to-body weight ratio in the trained groups [4.56 ± 0.13 (SED) vs. 5.29 ± 0.16 g (TRN) for females and 4.16 ± 0.11 (SED) vs. 4.77 ± 0.1 g (TRN) for males, *P* < 0.05]. The increased heart mass in trained rats was also mirrored by significantly elevated ventricular myocyte length in male and female rats ([Table 1](#)).

Table 1. Morphological parameters of sedentary and exercise-trained male and female rats

	SED		TRN	
	Male	Female	Male	Female
Body wt, g	421.4 \pm 13.0	253.6 \pm 3.1 [†]	384.4 \pm 5.6	249.8 \pm 5.5 [†]

Heart wt, mg	1,734.2 ± 50.1	1,122.5 ± 41.2 [†]	1,827 ± 62.4	1,326.4 ± 45.7 [†]
Heart wt/body wt, mg/g	4.16 ± 0.11	4.56 ± 0.13	4.77 ± 0.1 [*]	5.29 ± 0.16 [*]
Myocyte length, μm	87.34 ± 2.05	84.94 ± 1.6	103.08 ± 1.82 [*]	98.74 ± 2.02 [*]
Myocyte width, μm	22.58 ± 0.62	21.71 ± 0.56	24.35 ± 0.62	22.19 ± 0.53
<i>N</i>	15	15	15	15
<i>n</i>	102	100	112	108

Values are means ± SE. *N*, number of rats; *n*, number of myocytes. Myocyte length and width were not significantly different between heart regions, so data were pooled. SED, sedentary; TRN, exercise-trained.

^{*}*P* < 0.05 vs. SED;

[†]*P* < 0.05 vs. male.

AP and Ca²⁺ Transients

With the exception of the Ca²⁺ transient amplitude, no significant sex differences were observed. As a result, male and female data were pooled for [Figs. 2–5, 7, and 8](#) and are presented as sedentary and exercise-trained groups for clarity. [Figure 2A](#) shows a representative AP obtained at 1-Hz stimulation from left ventricular apex and base myocytes (representative of endocardial and epicardial myocytes, respectively) from control and trained rats. The AP was characterized by an initial spike potential followed by a plateau phase at ~20 mV and then repolarization. With 1-Hz stimulation, APD₉₀ values at 23°C were significantly longer in base (70 ± 2 ms in males and 76 ± 2 ms in females) than apex (57 ± 2 ms in males and 55 ± 1 ms in females) myocytes ([Fig. 2B](#)). The difference in APD₉₀ between the base and apex regions was also observed in myocytes from trained animals (85 ± 2 vs. 65 ± 1 ms in males and 88 ± 2 vs. 65 ± 1 ms in females). Apex and base APD₉₀ values were not different between males and females; however, wheel running significantly prolonged the APD₉₀ in both regions and sexes ([Fig. 2, A and B](#)).

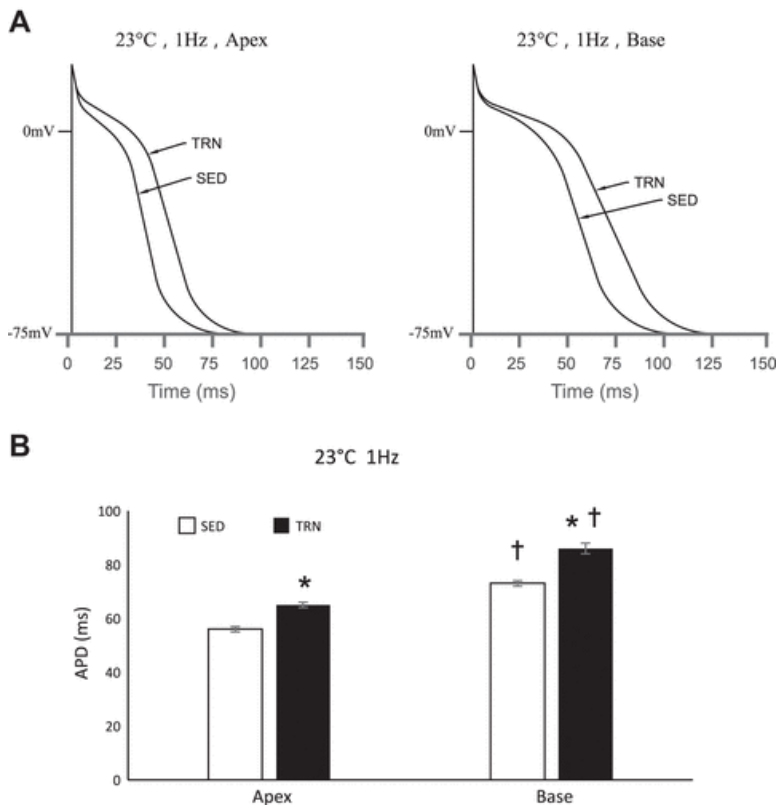


Fig. 2. Action potential (AP) duration (APD) measured at 90% repolarization (APD₉₀) in apex and base myocytes show regional difference, and APD₉₀ values for both are significantly prolonged by exercise training. *A*: overlapping representative

AP traces from apex and base myocytes from sedentary (SED) and exercise-trained (TRN) female rats. Measurements were obtained at room temperature with 1-Hz stimulation. *B*: APD₉₀ of myocytes from sedentary (apex: $N = 9$, $n = 36$, base: $N = 9$, $n = 34$) and exercise-trained (apex: $N = 8$, $n = 37$, base: $N = 8$, $n = 33$) rats. Values are means \pm SE. Data from male and female rats were pooled, as no significant sex difference was observed. $\dagger P < 0.05$ vs. apex. $*P < 0.01$ vs. SED.

Ca²⁺ transient duration monitored with fluo 4 (Fig. 3A) mirrored the regional differences and changes in APD₉₀ at 1-Hz stimulation, with exercise training significantly prolonging the transient in apex (Fig. 3B; 452 \pm 13 vs. 507 \pm 13 ms in males and 454 \pm 9 vs. 503 \pm 9 ms in females) and base (549 \pm 12 vs. 608 \pm 11 ms in males and 563 \pm 8 vs. 617 \pm 12 ms in females) myocytes. This training-induced prolongation was confirmed in measurements with fura 2 (data not shown).

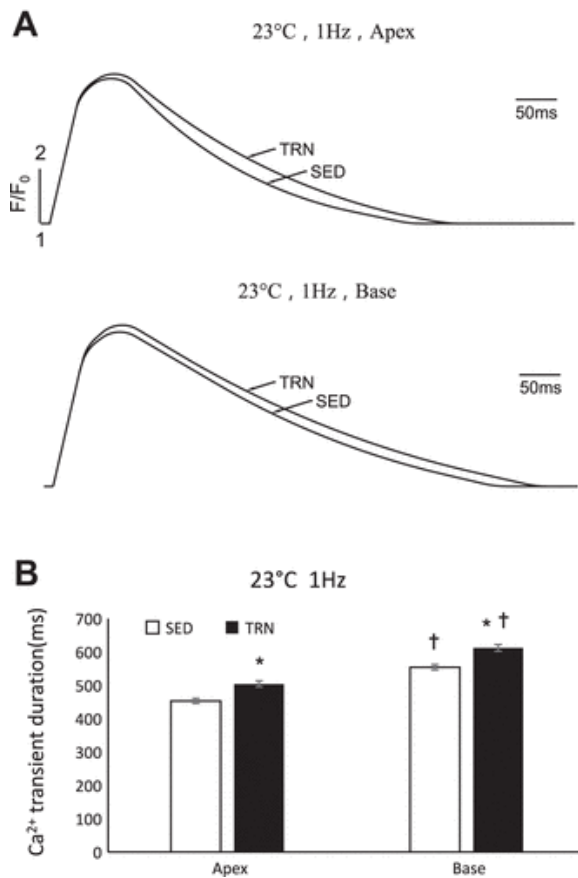


Fig. 3. Ca²⁺ transients monitored by fluo 4 correspond to regional differences and training-induced prolongation of the AP. *A*: simultaneous recordings of Ca²⁺ fluorescence from the same myocytes from which AP traces in Fig. 2A were obtained. Ca²⁺ signal amplitude is shown as fluorescence ratio (F/F₀), with fluorescence intensity (F) normalized to fluorescence intensity at rest before stimulation (F₀). *B*: base myocytes [$N = 9$, $n = 34$ (SED) and $N = 8$, $n = 33$ (TRN)] show longer Ca²⁺ transient durations than apex myocytes [$N = 9$, $n = 36$ (SED) and $N = 8$, $n = 37$ (TRN)]. At 1 Hz, exercise training prolonged the Ca²⁺ transient in base and apex myocytes. Values are means \pm SE. Data from male and female rats were pooled, as no significant sex difference was observed. $*P < 0.01$ vs. SED. $\dagger P < 0.05$ vs. apex.

The amplitude of the intracellular Ca²⁺ transient was determined from the fura 2 fluorescence intensity ratio at 340/380-nm excitation wavelength (Fig. 4A). Peak Ca²⁺ transient amplitudes at 1 Hz were smaller in myocytes from both sedentary and trained female than male rats in apex [Fig. 4B; 0.033 \pm 0.002 vs. 0.042 \pm 0.002 (SED) and 0.031 \pm 0.003 vs. 0.041 \pm 0.003 (TRN) ratio unit] and base [0.032 \pm 0.003 vs. 0.044 \pm 0.002 (SED) and 0.029 \pm 0.002 vs. 0.042 \pm 0.002 (TRN) ratio unit, $P < 0.05$] myocytes, with no significant exercise-training effect. The 10-

Hz, 60-s train significantly decreased peak Ca^{2+} transient amplitude and eliminated the sex differences (Fig. 4B), an effect associated with, and perhaps caused by, an elevated diastolic Ca^{2+} (Fig. 4C).

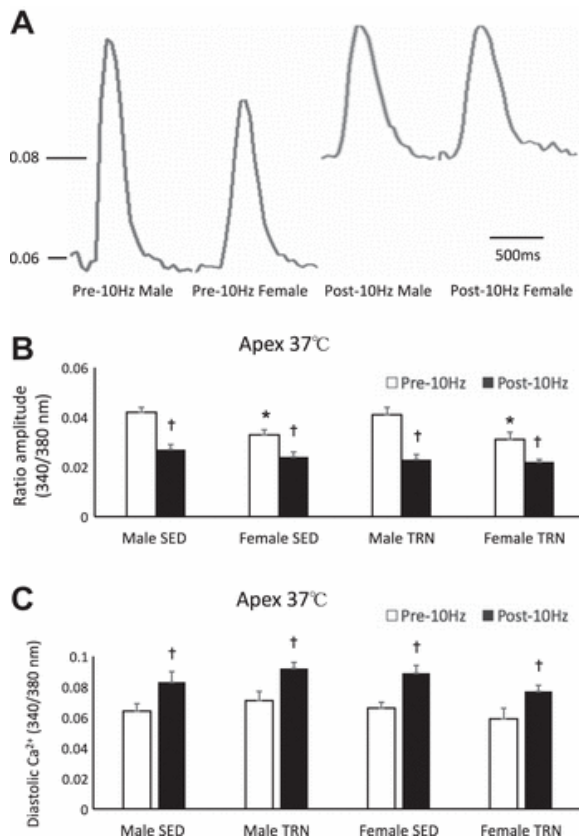


Fig. 4. A: representative 1-Hz Ca^{2+} transients (measured with fura 2) before (Pre) and immediately following (Post) a 10-Hz, 60-s train from the same base myocytes of sedentary male and female rats highlight the higher transient amplitude in males before, but not after, the 60-Hz train and the increased cytosolic Ca^{2+} in both sexes following the train. B: higher Ca^{2+} transient amplitude in myocytes from sedentary and exercise-trained male than female rats and no effect of exercise training on amplitude of the Ca^{2+} transient. C: 10-Hz train increased myocyte diastolic Ca^{2+} in both sexes and to the same extent in sedentary and trained rats. Data are from the apex region at 37°C; the same sex and stimulation rate effects were observed in the base region. Values are means \pm SE; $N = 3$, $n = 10\text{--}13$ for each group. * $P < 0.05$ vs. male. † $P < 0.05$ vs. Pre-10 Hz.

Temperature effects.

To assess myocyte adaptations at physiological temperatures, APs and Ca^{2+} transients were recorded at 37°C. Our results revealed that the exercise-training-induced lengthening of APD_{90} and the Ca^{2+} transient at 1-Hz stimulation remained at 37°C (Fig. 5), although both were significantly shortened at 37°C compared with 23°C (Fig. 5). The fluo 4 results (Fig. 5C) were confirmed with fura 2 (data not shown).

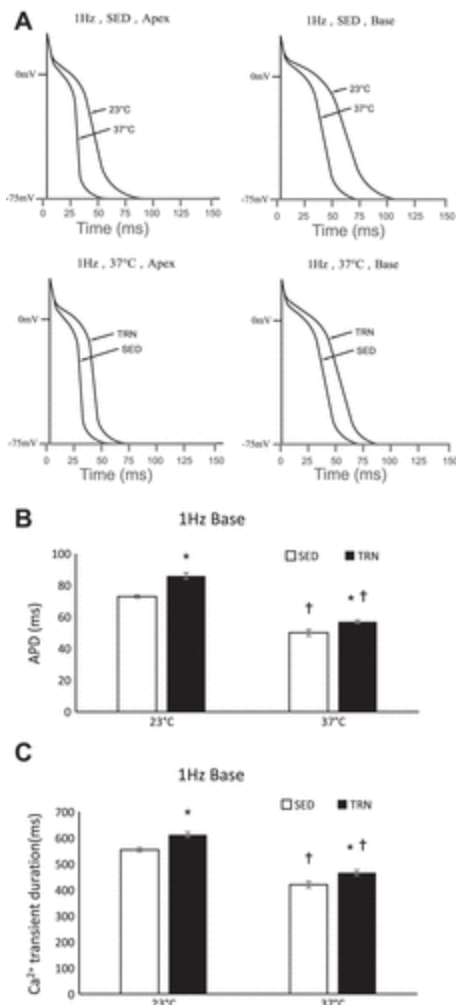


Fig. 5. APD₉₀ and Ca²⁺ transient duration were shortened at body temperature (37°C), with exercise-training effect still apparent. *A*: representative traces from apex and base regions showing temperature effect (*top*) and exercise-training-induced lengthening of APD at 37°C (*bottom*). *B*: APD₉₀ in myocytes from male and female rats were shortened by increasing temperature but remained longer in trained ($N = 8$, $n = 21$) than sedentary ($N = 9$, $n = 25$) rats. *C*: Ca²⁺transient duration (obtained with fluo 4) mirrored changes in APD at 37°C. Data are from the base region with 1-Hz stimulation; the same exercise-training and temperature effects were observed in the apex region. Values are means \pm SE. Data from male and female rats are pooled, as no significant sex difference was observed. * $P < 0.05$ vs. SED. † $P < 0.01$ vs. 23°C.

Activation rate effects.

To determine the effects of exercise training on myocyte adaptations at higher activation rates, we compared low (1-Hz) stimulation, where K_{ATP} channels would not be activated, with 5- and 10-Hz stimulation, representative of heart rates at rest and during heavy exercise, respectively (51). The 5-Hz experiments were performed on a subset of animals [$N = 4$, $n = 25$ (SED) and $N = 3$, $n = 17$ (TRN)] and, similar to the 1-Hz experiments, APDs were significantly prolonged in the wheel-trained rats in apex [52 ± 2 (SED) vs. 61 ± 3 (TRN) ms] and base [69 ± 3 (SED) vs. 80 ± 4 (TRN) ms] myocytes. In all groups, 10-Hz stimulation shortened APD₉₀ compared with 1-Hz stimulation (Fig. 6, A and B). Importantly, APD₉₀ was shortened significantly more in myocytes from exercise-trained than sedentary rats in apex [Fig. 6, B and C; $\Delta 28 \pm 2$ vs. $\Delta 15 \pm 3$ ms (male) and $\Delta 31 \pm 2$ vs. $\Delta 17 \pm 1$ ms (female)] and base [$\Delta 35 \pm 2$ vs. $\Delta 17 \pm 1$ ms (male) and $\Delta 33 \pm 3$ vs. $\Delta 21 \pm 1$ ms (female)] myocytes. Because of some summation of the Ca²⁺ transient during 10-Hz stimulation, we assessed the Ca²⁺ transient duration recorded at 1 Hz immediately following a 10-Hz, 60-s train. In myocytes from sedentary, but not trained, rats, the transients were significantly prolonged following the 10-Hz train in apex [Fig. 6C;

$\Delta 61 \pm 5$ vs. $\Delta 34 \pm 4$ ms (male) and $\Delta 57 \pm 5$ vs. $\Delta 26 \pm 3$ ms (female)] and base [$\Delta 69 \pm 6$ vs. $\Delta 42 \pm 5$ ms (male) and $\Delta 66 \pm 4$ vs. $\Delta 35 \pm 4$ ms (female)] myocytes.

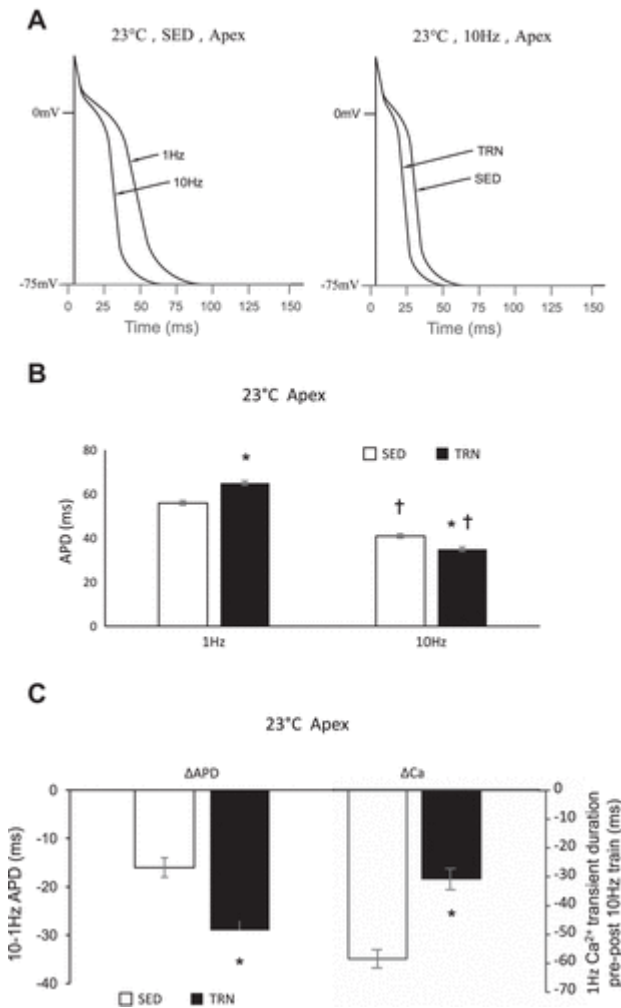


Fig. 6. At 10 Hz, APD₉₀ was shortened more in myocytes from exercise-trained than sedentary rats. *A*: representative AP traces highlighting high pulse rate-induced shortening (*left*) and exercise-training effect at 10 Hz (*right*). *B*: APD₉₀ is shortened more with 10-Hz stimulation in myocytes from exercise-trained ($N = 8$, $n = 23$) than sedentary ($N = 9$, $n = 33$) rats. *C*: 10 Hz elicited greater shortening of the myocyte APD in exercise-trained than sedentary rats (*left*), and after a 10-Hz, 60-s train, Ca²⁺ transients (monitored with fluo 4) elicited by a 1-Hz pulse increased to a greater extent in myocytes from sedentary ($N = 9$, $n = 33$) than exercise-trained ($N = 8$, $n = 23$) rats. Data in *B* and *C* are from apex myocytes at 23°C; the same exercise-training effect was observed in base myocytes. Values are means \pm SE. Data from male and female rats are pooled, as no significant sex difference was observed. * $P < 0.05$ vs. SED. † $P < 0.01$ vs. 1 Hz.

Training Effect on the K_{ATP} Channel

The K_{ATP} inhibitor glibenclamide (2 μ M) prolonged APD₉₀ at 10 Hz. The prolongation was significantly greater in myocytes from exercise-trained [apex: $\Delta 17 \pm 3$ ms (male) and $\Delta 23 \pm 4$ ms (female); base: $\Delta 11 \pm 2$ ms (male) and $\Delta 15 \pm 3$ ms (female)] than control [apex: $\Delta 10 \pm 3$ ms (male) and $\Delta 15 \pm 3$ ms (female); base: $\Delta 7 \pm 1$ ms (male) and $\Delta 10 \pm 2$ ms (female)] rats. Regional differences in glibenclamide-induced prolongation were also observed in trained male and female rats, with greater changes in apex than base myocytes ([Fig. 7](#)).

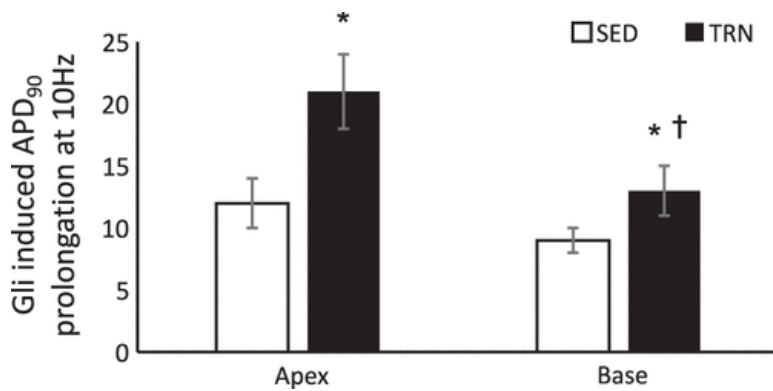


Fig. 7. At 10 Hz, APD₉₀ was prolonged more by the K_{ATP} channel inhibitor glibenclamide (Gli) in myocytes from exercise-trained than sedentary rats. APD₉₀ differences before and after administration of 2 μM glibenclamide with 10-Hz stimulation were significantly greater in myocytes from exercise-trained than sedentary rats. In myocytes from exercise-trained rats, glibenclamide-induced APD₉₀prolongation was greater in the apex than base region. Values are means ± SE; *N* = 6, *n* = 32–38 per group. Data from male and female rats are pooled, as no significant sex difference was observed. **P* < 0.05 vs. SED. †*P* < 0.05 vs. apex.

Training Effect on β-AR Responsiveness

Administration of the nonselective β-AR antagonist propranolol (10 μM) showed no effect on APD₉₀ in exercise-trained [apex: 65 ± 1 vs. 66 ± 3 ms (male) and 65 ± 1 vs. 65 ± 2 ms (female); base: 85 ± 2 vs. 83 ± 2 ms (male) and 88 ± 2 vs. 87 ± 3 ms (female)] or sedentary [apex: 57 ± 2 vs. 59 ± 3 ms (male) and 55 ± 1 vs. 53 ± 2 ms (female); base: 70 ± 2 vs. 68 ± 3 ms (male) and 76 ± 1 vs. 77 ± 3 ms (female)] groups (*N* = 3, *n* = 11–14 per group). [Figure 8A](#) shows the dose-response relationship of the nonselective β-AR agonist isoproterenol to APD₉₀, expressed as a percent decrease (%change relative to APD₉₀ without agonist). Wheel running decreased the logIC₅₀ (the concentration giving a half-maximal effect) in male [−8.9 vs. −8.2 (apex) and −8.8 vs. −8.2 (base)] and female [−9 vs. −8.1 (apex) and −8.8 vs. −8.1 (base)] rats, such that between 0.1 and 100 nM, the drug-response curve shifted rightward with exercise training by ~1 log unit compared with controls. Thus, myocytes from wheel-running rats required higher isoproterenol levels to produce reductions in APD₉₀ similar to those observed in myocytes from sedentary rats.

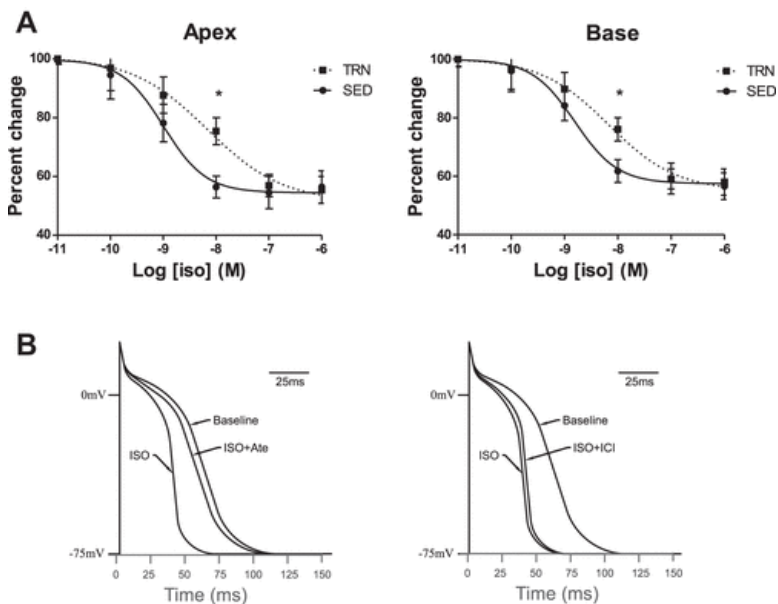


Fig. 8. Dose-response relationship of the nonselective β -adrenergic receptor (AR) agonist isoproterenol (Iso) and effect of the selective β_1 -AR antagonist atenolol (Ate) and the β_2 -AR blocker ICI 118,551 (ICI) on isoproterenol-induced APD₉₀ shortening. A: measurements obtained from myocytes superfused for >2 min at each isoproterenol concentration from 10^{-11} to 10^{-6} M, expressed as %decrease (APD₉₀with agonist/APD₉₀ without agonist); values for APD₉₀ without agonist are from Fig. 3. Exercise training shifted the curve rightward by ~1 log unit in all tested groups. Values are means \pm SE; $N = 8$, $n = 20$ –24 per group. Data from male and female rats are pooled, as no significant sex difference was observed. * $P < 0.05$, TRN vs. SED. B: representative AP recordings in myocytes from the base region of a sedentary male rat with 1-Hz stimulation at room temperature before addition of agonist or blocker (baseline), after administration of 1 μ M isoproterenol alone and 1 μ M isoproterenol + 100 nM atenolol (left), or, in a separate myocyte, in the presence of 1 μ M isoproterenol + 100 nM ICI 118,551 (right).

To distinguish the training effect on β -AR subtypes, myocytes were stimulated at 1 Hz during exposure to 1 μ M isoproterenol (the concentration that elicited the peak response) in the presence of 0.1 μ M ICI 118,551 (a selective β_2 -AR antagonist) or 0.1 μ M atenolol (a selective β_1 -AR antagonist). Myocyte APD₉₀ showed little response to addition of the β_2 -AR blocker in the sedentary [apex: 32 ± 2 vs. 34 ± 3 ms (male) and 30 ± 1 vs. 33 ± 1 ms (female); base: 40 ± 1 vs. 44 ± 3 ms (male) and 45 ± 2 vs. 48 ± 3 ms (female)] and exercise-trained [apex: 39 ± 2 vs. 43 ± 2 ms (male) and 37 ± 2 vs. 40 ± 3 ms (female); base: 47 ± 3 vs. 52 ± 3 ms (male) and 52 ± 2 vs. 56 ± 3 ms (female)] groups. In contrast, the selective β_1 -AR blocker largely reversed the isoproterenol-induced shortening of APD₉₀ (Fig. 8B). This reversal occurred in myocytes from sedentary [apex: $\Delta 23 \pm 4$ ms (male) and $\Delta 21 \pm 5$ ms (female); base: $\Delta 26 \pm 5$ ms (male) and $\Delta 25 \pm 3$ ms (female)] and trained [apex: $\Delta 24 \pm 4$ ms (male) and $\Delta 25 \pm 3$ ms (female); base: $\Delta 30 \pm 5$ ms (male) and $\Delta 31 \pm 4$ ms (female)] groups ($N = 3$, $n = 10$ –14 per group for each blocker tested). This suggests that much of the exercise-training-altered responsiveness of APD₉₀ to adrenergic activation is mediated through β_1 -ARs.

When myocytes were exposed to the maximal-response dose of isoproterenol (1 μ M) and stimulated at 10 Hz, both β -AR and K_{ATP} activation contributed to APD shortening. Figure 9 shows the relative importance of β -AR and K_{ATP} together with other unknown factors in different regions from sedentary male and female rats. In both regions and sexes, β -AR stimulation produced the greatest effect [apex $57 \pm 15\%$ (male) and $50 \pm 12\%$ (female); base: $61 \pm 17\%$ (male) and $58 \pm 14\%$ (female)]; however, the contribution of the K_{ATP} channel was still important [apex: $23 \pm 11\%$ (male) and $38 \pm 10\%$ (female); base: $14 \pm 7\%$ (male) and $19 \pm 8\%$ (female)]. No significant differences were observed between the apex and base regions for male and female groups. While this sequence of activation was not performed in myocytes from trained rats, their higher K_{ATP} channel function should increase the contribution of this channel to APD shortening relative to controls.

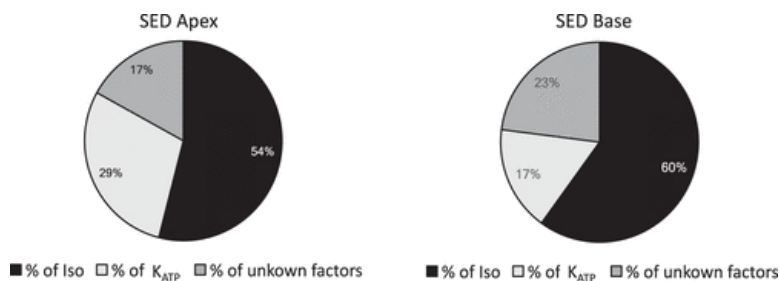


Fig. 9. Relative contribution of β -AR stimulation and K_{ATP} channel activation in myocytes from apex and base regions of sedentary male and female rats. Three-section pie charts were plotted to highlight effects of β -AR and K_{ATP} channel on APD shortening at 10 Hz. “% of unknown factors” represents missing proportions. β -AR activation has a larger effect than K_{ATP} channel opening in all tested groups ($N = 6$, $n = 24$ –26 per group). Data from male and female rats are pooled, as no significant sex difference was observed.

DISCUSSION

Exercise-training-induced cardioprotection is mediated in part by improving the electrical properties and, in turn, the force of the heart (35, 40, 57). The heartbeat is initiated by a pulse of electrical excitation, the cardiac AP. It is determined by the interplay of voltage-gated Na⁺, Ca²⁺, and K⁺ channels. After activation, cardiomyocytes remain depolarized (the AP plateau) due to Ca²⁺ influx via the L-type voltage-gated Ca²⁺ channels. The Ca²⁺ influx triggers Ca²⁺ release from the SR, and the resulting Ca²⁺ transients initiate myocyte contractions. Myocytes repolarize due to activation of delayed rectifier and K_{ATP} channels, returning the transmembrane potential to the initial resting level and, thereby, allowing myocardial relaxation and ventricular filling. Thus the excitation-contraction coupling process is closely regulated by the AP plateau and the overall AP and Ca²⁺ transient durations. Importantly, in this work we showed that regular exercise training prolonged myocyte AP and Ca²⁺ transient durations in both sexes and regions of the left ventricle at low activation (1-Hz stimulation) and shortened AP and Ca²⁺ transient durations at high (10-Hz) stimulation. A novel observation was that these responses to training were mediated (at least in part) by both altered β₁-AR regulation and increased K_{ATP} channel function. These adaptations help explain the increased efficiency and contractility induced by regular exercise training (20).

Wheel Running as an Exercise-Training Paradigm

Voluntary wheel running produced desirable changes in the APD and, similar to treadmill running, increased the heart weight-to-body weight ratio, but without the increase in stress that complicates the interpretation of enforced, intense exercise regimens (3, 32, 35). The extent and pattern of wheel running in our study were consistent with those used in previous studies (13, 29). Our data showed that the female rats ran almost twice the distance run by the male rats and that this greater distance was in part due to higher running speed. The explanation for higher running speeds in females is unknown, but it could be related to sex hormones and the estrous cycle (9, 15, 21). Despite a large difference in the distance run, no differences in cardiac hypertrophy were observed between male and female rats. Our observation that regular exercise increased myocyte length, but not width, provided additional morphological evidence that training elicited left ventricular cardiac hypertrophy and agreed with the findings of Mokolke et al. (31) and Palmer et al. (38) that treadmill running in female rats produced significant increases in mean myocyte capacitance and length, but not myocyte width. These results suggest that the exercise-training-induced cardiac hypertrophy, unlike pressure overload hypertrophy, is due to increased cell length, rather than width (20). Similar levels of cellular hypertrophy were reported for wheel-trained rats by others without consideration of sex differences (35, 53, 54). However, there may be species differences in susceptibility to hypertrophy, as sex differences in exercise-induced myocardial hypertrophy were observed in mice, with females showing greater hypertrophy than males (15, 27). Besides cardiac hypertrophy, exercise-training programs using wheel and treadmill running both significantly decrease resting heart rate and increase limb muscle respiratory capacity (17, 51).

Effect of Exercise Training on APD and the Ca²⁺ Transient

A number of studies have shown transmural differences in the AP shape; APD has been shown to be longer in endocardial than epicardial myocytes (10, 11, 35, 40). Since ventricular activation initiates in the endocardium and spreads transversely, endocardial myocytes depolarize first but, because of their longer APD, repolarize after epicardial cells. This allows the endocardial cells to remain contracted during epicardial contraction, which may aid ventricular ejection and prevent arrhythmias caused by reexcitation of endocardial myocytes (43).

There are few studies of exercise-training-induced adaptation in the APD other than a report that, in female rats, wheel running reduced the transmural difference in APD by lengthening the epicardial APD (35). Our results are consistent with the regional differences reported by others. However, our wheel-training results differ from those of Natali et al. (35), in that we observed an exercise-induced prolongation of myocyte APD in cells isolated

from both the apex and base regions of male and female hearts, while their study of females showed a prolongation only in epicardial cells. The reason for this discrepancy is unknown but could be related to how myocytes representative of epicardial and endocardial cells were isolated. Natali et al. (35) harvested the cells from subepicardial and subendocardial layers, while we assumed that cells from the base and apex primarily reflected endocardial and epicardial myocytes (10). To resolve the discrepancy, future studies should examine subendocardial and subepicardial cell layers in the base and apex regions separately. Another difference between the study of Natali et al. (35) and our study is that the AP recorded in our laboratory showed a plateau above 0 mV, while their AP plateaued at approximately -30 mV. This negative potential may have reduced K⁺ repolarizing currents, which would be consistent with the longer APD₉₀ of sedentary females (76.2 ± 4.4 and 107.8 ± 6.9 ms for epicardial and endocardial layers, respectively) recorded by Natali et al. (35). The plateau level and overall length of APs presented in our study are consistent with those reported by others (1, 42). For example, in their study of sedentary male rats, Sun et al. (42) showed that ventricular cell APs at 25°C plateaued above 0 mV and had an APD₉₀ of 51.3 ± 8.3 ms.

With 1-Hz stimulation, the Ca²⁺ transient shape and duration measured with fura 2 and fluo 4 are consistent with previous studies on rat ventricular myocytes (1, 22) and mirrored the regional differences and exercise-training effect in APD₉₀. As expected, Ca²⁺ transient durations measured with fura 2 and fluo 4 were significantly shortened at 37°C compared with 23°C, as the higher temperature would accelerate the kinetics of sarcolemma ion channels and SR Ca²⁺ release and reuptake. Nonetheless, the exercise-training effects remained apparent at the higher temperature, suggesting that exercise training either increased Ca²⁺ inward current or decreased K⁺ repolarizing currents at low activation rates. Mokolke et al. reported that endurance exercise training had no effect on L-type Ca²⁺ channel number or Ca²⁺ current characteristics (31). Less is known about how exercise training alters the various K⁺ channels. These channels include the rapidly activating, transient I_{to} channel, which generates the early repolarization preceding the AP plateau, and the delayed rectifier K⁺ (I_k) channels, which are important in repolarization following the AP plateau (24, 25). Stones et al. (40) observed that 6 wk of wheel-running exercise in rats reduced the I_{to} current density in epicardial myocytes and suggested that this adaptation could explain the prolonged monophasic AP of epicardial cells on the heart's surface. Clearly, the possibility that K⁺ channels might be involved in adaptation to exercise training requires further investigation.

We evaluated the effects of wheel running on myocyte APD₉₀ with 5- and 10-Hz stimulation, as these rates are reflective of rat heart rates under rest and maximal-exercise conditions, respectively (51). At 5 Hz, similar to 1 Hz, the exercise training prolonged APD₉₀. This suggests that myocyte activation rates under resting conditions are insufficient to open the K_{ATP} channel or increase the β-AR agonist enough to override the reduced response of the β₁-AR to the agonist. In contrast, the 10-Hz stimulation rate shortened the APD₉₀ compared with 1 and 5 Hz in all conditions (including base/apex, male/female, and trained/control), with APD₉₀ shortened more in myocytes from exercise-trained than sedentary male and female rats. This suggests that the exercise training increased K⁺ repolarizing currents at the higher activation rate. This could result from an exercise-training-induced increase in the delayed rectifier and/or K_{ATP} channels activated by reduced ATP-to-ADP ratio, when metabolic demand exceeds oxygen supply (25, 37). One effect of a higher K⁺ efflux and faster AP repolarization during heavy exercise would be to limit Ca²⁺ entry into the cell, which would reduce energy requirements for ion homeostasis and contraction, speed relaxation, and spare time for ventricular filling. This is supported by our observation that exercise training eliminated the Ca²⁺ transient prolongation observed after the 10-Hz, 60-s stimulation in myocytes from sedentary rats. This suggests that myocytes from the trained animals were more effective in Ca²⁺ removal, presumably because of increased SERCA pump activity (8, 26, 49). Nevertheless, 10-Hz stimulation elevated diastolic Ca²⁺ in myocytes from both sedentary and trained rats, which suggests that wheel running was unable to sufficiently increase the intracellular Ca²⁺ removal processes.

With 1-Hz stimulation, we observed smaller peak Ca^{2+} transient amplitudes in myocytes from both trained and sedentary females than males. This sex difference was abolished when myocytes were activated at 1 Hz directly following a 10-Hz, 60-s train, a stimulation pattern that elevated diastolic Ca^{2+} to a similar degree in all tested groups. The observation of a sex difference in Ca^{2+} transient amplitude agrees with the report of Farrell et al. (16) of a reduced amplitude and duration of the unitary (spark) SR Ca^{2+} release in myocytes from females compared with males. Our finding that wheel running caused no significant alteration in the amplitude of the Ca^{2+} transient agrees with the findings of Natali et al. (35) and Stones et al. (41) and the observation that L-type Ca^{2+} current was unaltered by voluntary exercise (31).

Exercise Training Effect on K_{ATP} Channel and β -AR Responsiveness

Our observation that the K_{ATP} channel blocker glibenclamide caused a significantly greater prolongation of APD during 10-Hz stimulation in myocytes from trained than sedentary rats agrees with the reports of Zingman et al. (57) that as few as 5 days of treadmill running increased K_{ATP} channel expression in mouse hearts and Brown et al. (7) that myocyte K_{ATP} content increased following a 12-wk treadmill-running program in female rats. While 10-Hz stimulation shortened both the apex and base myocyte APD the same amount, glibenclamide induced greater prolongation in apex than base myocytes from trained rats. This suggests that wheel running increased K_{ATP} channel function to a greater extent in the apex and that other training-induced adaptations, in addition to K_{ATP} regulation, contributed to the reduced APD during 10-Hz stimulation.

Repolarizing K^+ currents are known to be regulated by the sympathetic nervous system (44, 45), and exercise training is thought to decrease sympathetic tone at rest (4, 48). However, our observation that the nonselective β -AR antagonist propranolol had no effect on APD_{90} in myocytes from any group rules out the possibility that the prolonged APD_{90} at 1 Hz following wheel running was an effect of endogenous β -AR agonist remaining in the cells following isolation. However, administration of the mixed β -AR agonist isoproterenol shortened APD_{90} , likely because of increased repolarizing K^+ currents. It has been suggested that α -AR agonists inhibit repolarizing currents and prolong ventricular APD in isolated cardiomyocytes (5, 46), while β -AR activity increases outward current amplitude (23, 46). Although the maximal isoproterenol-induced shortening of APD_{90} was comparable in sedentary and trained rats, wheel running decreased the responsiveness of β -AR stimulation by shifting the drug-response curve to the right. Thus, for similar acceleration of the AP, myocytes from trained rats require higher levels of isoproterenol than myocytes from sedentary rats. This could partially explain the observation that wheel running prolonged monophasic AP in beating rat hearts (40), as a reduced myocyte response to a β -AR agonist would allow α -AR-mediated inhibition of K^+ currents to predominate, thus lengthening APD (46, 55). The reduced β -AR responsiveness can also explain the well-described reduction in resting heart rate in exercise-trained animals (51).

By applying a combination of subtype-selective blockers and a maximum dose of isoproterenol, we distinguished whether reduced myocyte AP responsiveness to β -AR regulation induced by exercise training was mediated through β_1 - or β_2 -ARs. Stone et al. (41) showed that voluntary wheel running reduced the myocyte inotropic response to β_2 -AR, but not β_1 -AR, stimulation. However, our observation of little response to the addition of a β_2 -AR blocker revealed that modulation of β_2 -AR responsiveness to adrenergic regulation of APD is not a major adaptive mechanism in the response to voluntary exercise, while isoproterenol-induced AP shortening was largely reversed by the selective β_1 -AR blocker comparably in base and apex regions. Our results are consistent with the finding of Barbier et al. (4) that β_1 -AR, but not β_2 -AR, protein expression was decreased with exercise training. These results suggest that exercise training affects adrenergic regulation of APD by reducing primarily β_1 -AR responsiveness and that this response can, at least in part, explain the exercise-training-induced increase in left ventricular APD at the whole heart level (40).

Our results demonstrate that while the β -AR mechanism is quantitatively most important in reducing APD at high activation rates, both the β -AR and K_{ATP} channel play important roles. The observation that the K_{ATP} channel inhibitor glibenclamide prolonged APD when applied to a cell contracting at 10 Hz in the presence of a saturating dose of the β -AR agonist isoproterenol demonstrates the importance of the K_{ATP} channel in facilitating ventricular repolarization, even in the presence of maximal sympathetic nervous system drive (i.e., β -AR activation), and validated the importance of both β -AR and K_{ATP} channel to the training-induced adaptations in myocyte function. Since changes in the responsiveness of adrenergic regulation cannot explain the prolongation of APD (at 1 and 5 Hz) induced by exercise training in isolated myocytes, alterations of sarcolemma K^+ channels independent of adrenergic factors must play a role in the training adaptation. To elucidate these factors, future studies will aim to determine the extent to which wheel training alters the kinetics, voltage dependence, current density, and expression level of repolarizing K^+ currents, including I_K and K_{ATP} channels.

In summary, exercise training significantly increased APD measured at 1 and 5 Hz while decreasing APD at 10 Hz. Ca^{2+} transient durations reflected the changes in APD. The exercise program elevated the K_{ATP} channel contribution to AP shortening at 10 Hz, with greater adaptation in apex than base myocytes. Wheel running reduced the sensitivity of the myocytes to isoproterenol by shifting the dose-response curve rightward compared with controls, with a selective alteration of β_1 -AR responsiveness as the underlying mechanism. These exercise-training adaptations should contribute to enhanced cardiac contractility and efficiency at low heart rates by prolonging the duration of the Ca^{2+} transient and reducing the energy requirements for ion homeostasis. In contrast, the shortened APD at high work rates or in stress conditions should maintain a diastolic interval adequate for myocardial relaxation.

GRANTS

This work was supported by American Heart Association Grant 16PRE31090010 (to X. Wang) and a Marquette University Department of Biological Sciences Committee on Research Grant and a Marquette University Way-Klingler Fellowship (to R. H. Fitts).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

X.W. and R.H.F. conceived and designed research; X.W. performed experiments; X.W. analyzed data; X.W. and R.H.F. interpreted results of experiments; X.W. prepared figures; X.W. drafted manuscript; X.W. and R.H.F. approved final version of manuscript; R.H.F. edited and revised manuscript.

ACKNOWLEDGMENTS

We thank Dr. Mehdi Maadooliat (Marquette University) for assistance with statistical analysis. We are grateful to Dr. Wai-Meng Kwok (Medical College of Wisconsin) for technical support and reading an earlier version of the manuscript and Christopher W. Sundberg (Marquette University) for help with manuscript preparation.

AUTHOR NOTES

- Address for reprint requests and other correspondence: R. H. Fitts, 530 N. 15th St. WLS Bldg. B021, Milwaukee, WI 53233 (e-mail: robert.fitts@marquette.edu).

REFERENCES

1. **Al Kury LT, Voitychuk OI, Ali RM, Galadari S, Yang KH, Howarth FC, Shuba YM, Oz M.** Effects of endogenous cannabinoid anandamide on excitation-contraction coupling in rat ventricular myocytes. *Cell Calcium* 55: 104–118, 2014. doi:[10.1016/j.ceca.2013.12.005](https://doi.org/10.1016/j.ceca.2013.12.005).
2. **Amin AS, Pinto YM, Wilde AA.** Long QT syndrome: beyond the causal mutation. *J Physiol* 591: 4125–4139, 2013. doi:[10.1113/jphysiol.2013.254920](https://doi.org/10.1113/jphysiol.2013.254920).
3. **Asami S, Hirano T, Yamaguchi R, Itoh H, Kasai H.** Reduction of 8-hydroxyguanine in human leukocyte DNA by physical exercise. *Free Radic Res* 29: 581–584, 1998. doi:[10.1080/10715769800300621](https://doi.org/10.1080/10715769800300621).
4. **Barbier J, Rannou-Bekono F, Marchais J, Berthon PM, Delamarche P, Carré F.** Effect of training on $\beta_1\beta_2\beta_3$ -adrenergic and M2 muscarinic receptors in rat heart. *Med Sci Sports Exerc* 36: 949–954, 2004. doi:[10.1249/01.MSS.0000128143.93407.39](https://doi.org/10.1249/01.MSS.0000128143.93407.39).
5. **Bian J, Cui J, McDonald TV.** HERG K⁺ channel activity is regulated by changes in phosphatidylinositol 4,5-bisphosphate. *Circ Res* 89: 1168–1176, 2001. doi:[10.1161/hh2401.101375](https://doi.org/10.1161/hh2401.101375).
6. **Billman GE.** Cardiac autonomic neural remodeling and susceptibility to sudden cardiac death: effect of endurance exercise training. *Am J Physiol Heart Circ Physiol* 297: H1171–H1193, 2009. doi:[10.1152/ajpheart.00534.2009](https://doi.org/10.1152/ajpheart.00534.2009).
7. **Brown DA, Chicco AJ, Jew KN, Johnson MS, Lynch JM, Watson PA, Moore RL.** Cardioprotection afforded by chronic exercise is mediated by the sarcolemmal, and not the mitochondrial, isoform of the K_{ATP} channel in the rat. *J Physiol* 569: 913–924, 2005. doi:[10.1113/jphysiol.2005.095729](https://doi.org/10.1113/jphysiol.2005.095729).
8. **Carneiro-Júnior MA, Prímola-Gomes TN, Quintão-Júnior JF, Drummond LR, Lavorato VN, Drummond FR, Felix LB, Oliveira EM, Cruz JS, Natali AJ, Mill JG.** Regional effects of low-intensity endurance training on structural and mechanical properties of rat ventricular myocytes. *J Appl Physiol (1985)* 115: 107–115, 2013. doi:[10.1152/jappphysiol.00041.2013](https://doi.org/10.1152/jappphysiol.00041.2013).
9. **Chatterton RT Jr, Hrycyk L, Hickson RC.** Effect of endurance exercise on ovulation in the rat. *Med Sci Sports Exerc* 27: 1509–1515, 1995. doi:[10.1249/00005768-199511000-00007](https://doi.org/10.1249/00005768-199511000-00007).
10. **Clark RB, Bouchard RA, Salinas-Stefanon E, Sanchez-Chapula J, Giles WR.** Heterogeneity of action potential waveforms and potassium currents in rat ventricle. *Cardiovasc Res* 27: 1795–1799, 1993. doi:[10.1093/cvr/27.10.1795](https://doi.org/10.1093/cvr/27.10.1795).
11. **Colli Franzone P, Pavarino LF, Scacchi S, Taccardi B.** Modeling ventricular repolarization: effects of transmural and apex-to-base heterogeneities in action potential durations. *Math Biosci* 214: 140–152, 2008. doi:[10.1016/j.mbs.2008.06.006](https://doi.org/10.1016/j.mbs.2008.06.006).
12. **Conrath CE, Opthof T.** Ventricular repolarization: an overview of (patho)physiology, sympathetic effects and genetic aspects. *Prog Biophys Mol Biol* 92: 269–307, 2006. doi:[10.1016/j.pbiomolbio.2005.05.009](https://doi.org/10.1016/j.pbiomolbio.2005.05.009).
13. **Craft RM, Clark JL, Hart SP, Pinckney MK.** Sex differences in locomotor effects of morphine in the rat. *Pharmacol Biochem Behav* 85: 850–858, 2006. doi:[10.1016/j.pbb.2006.11.022](https://doi.org/10.1016/j.pbb.2006.11.022).
14. **Dukes ID, Vaughan Williams EM.** Effects of selective α_1 , α_2 , β_1 , and β_2 -adrenoceptor stimulation on potentials and contractions in the rabbit heart. *J Physiol* 355: 523–546, 1984. doi:[10.1113/jphysiol.1984.sp015436](https://doi.org/10.1113/jphysiol.1984.sp015436).
15. **Dworatzek E, Mahmoodzadeh S, Schubert C, Westphal C, Leber J, Kusch A, Kararigas G, Fliegner D, Moulin M, Ventura-Clapier R, Gustafsson JA, Davidson MM, Dragun D, Regitz-Zagrosek V.** Sex differences in exercise-induced physiological myocardial hypertrophy are modulated by oestrogen receptor- β . *Cardiovasc Res* 102: 418–428, 2014. doi:[10.1093/cvr/cvu065](https://doi.org/10.1093/cvr/cvu065).
16. **Farrell SR, Ross JL, Howlett SE.** Sex differences in mechanisms of cardiac excitation-contraction coupling in rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* 299: H36–H45, 2010. doi:[10.1152/ajpheart.00299.2010](https://doi.org/10.1152/ajpheart.00299.2010).
17. **Fitts RH, Booth FW, Winder WW, Holloszy JO.** Skeletal muscle respiratory capacity, endurance, and glycogen utilization. *Am J Physiol* 228: 1029–1033, 1975.
18. **Fletcher GF, Balady G, Blair SN, Blumenthal J, Caspersen C, Chaitman B, Epstein S, Sivarajan Froelicher ES, Froelicher VF, Pina IL, Pollock ML.** Statement on exercise: benefits and recommendations for physical activity programs for all Americans. A statement for health professionals by the Committee on

Exercise and Cardiac Rehabilitation of the Council on Clinical Cardiology, American Heart Association. *Circulation* 94: 857–862, 1996. doi:[10.1161/01.CIR.94.4.857](https://doi.org/10.1161/01.CIR.94.4.857).

19. **Gassmayr S, Stadnicka A, Suzuki A, Kwok WM, Bosnjak ZJ.** Isoflurane sensitizes the cardiac sarcolemmal adenosine triphosphate-sensitive potassium channel to pinacidil. *Anesthesiology* 98: 114–120, 2003. doi: [10.1097/0000542-200301000-00020](https://doi.org/10.1097/0000542-200301000-00020).
20. **Gielen S, Schuler G, Adams V.** Cardiovascular effects of exercise training: molecular mechanisms. *Circulation* 122: 1221–1238, 2010. doi: [10.1161/CIRCULATIONAHA.110.939959](https://doi.org/10.1161/CIRCULATIONAHA.110.939959).
21. **Gorzek JF, Hendrickson KC, Forstner JP, Rixen JL, Moran AL, Lowe DA.** Estradiol and tamoxifen reverse ovariectomy-induced physical inactivity in mice. *Med Sci Sports Exerc* 39: 248–256, 2007. doi:[10.1249/01.mss.0000241649.15006.b8](https://doi.org/10.1249/01.mss.0000241649.15006.b8).
22. **Hamouda NN, Sydorenko V, Qureshi MA, Alkaabi JM, Oz M, Howarth FC.** Dapagliflozin reduces the amplitude of shortening and Ca²⁺ transient in ventricular myocytes from streptozotocin-induced diabetic rats. *Mol Cell Biochem* 400: 57–68, 2015. doi:[10.1007/s11010-014-2262-5](https://doi.org/10.1007/s11010-014-2262-5).
23. **Heath BM, Terrar DA.** Protein kinase C enhances the rapidly activating delayed rectifier potassium current, I_{Kr}, through a reduction in C-type inactivation in guinea-pig ventricular myocytes. *J Physiol* 522: 391–402, 2000. doi:[10.1111/j.1469-7793.2000.t01-2-00391.x](https://doi.org/10.1111/j.1469-7793.2000.t01-2-00391.x).
24. **Himmel HM, Wettwer E, Li Q, Ravens U.** Four different components contribute to outward current in rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* 277: H107–H118, 1999.
25. **Jew KN, Olsson MC, Mokolke EA, Palmer BM, Moore RL.** Endurance training alters outward K⁺ current characteristics in rat cardiocytes. *J Appl Physiol (1985)* 90: 1327–1333, 2001.
26. **Kemi OJ, Ellingsen O, Smith GL, Wisloff U.** Exercise-induced changes in calcium handling in left ventricular cardiomyocytes. *Front Biosci* 13: 356–368, 2008. doi:[10.2741/2685](https://doi.org/10.2741/2685).
27. **Konhilas JP, Maass AH, Luckey SW, Stauffer BL, Olson EN, Leinwand LA.** Sex modifies exercise and cardiac adaptation in mice. *Am J Physiol Heart Circ Physiol* 287: H2768–H2776, 2004. doi:[10.1152/ajpheart.00292.2004](https://doi.org/10.1152/ajpheart.00292.2004).
28. **Lehmann MH, Hardy S, Archibald D, quart B, MacNeil DJ.** Sex difference in risk of torsade de pointes with *d,l*-sotalol. *Circulation* 94: 2535–2541, 1996. doi:[10.1161/01.CIR.94.10.2535](https://doi.org/10.1161/01.CIR.94.10.2535).
29. **Lightfoot JT.** Sex hormones' regulation of rodent physical activity: a review. *Int J Biol Sci* 4: 126–132, 2008. doi:[10.7150/ijbs.4.126](https://doi.org/10.7150/ijbs.4.126).
30. **Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS.** Requirement of a macromolecular signaling complex for β -adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* 295: 496–499, 2002. doi:[10.1126/science.1066843](https://doi.org/10.1126/science.1066843).
31. **Mokolke EA, Palmer BM, Cheung JY, Moore RL.** Endurance training does not affect intrinsic calcium current characteristics in rat myocardium. *Am J Physiol Heart Circ Physiol* 273: H1193–H1197, 1997.
32. **Moraska A, Deak T, Spencer RL, Roth D, Fleshner M.** Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol* 279: R1321–R1329, 2000.
33. **Motomura S, Reinhard-Zerkowski H, Daul A, Brodde OE.** On the physiologic role of β_2 -adrenoceptors in the human heart: in vitro and in vivo studies. *Am Heart J* 119: 608–619, 1990. doi:[10.1016/S0002-8703\(05\)80284-4](https://doi.org/10.1016/S0002-8703(05)80284-4).
34. **Natali AJ, Turner DL, Harrison SM, White E.** Regional effects of voluntary exercise on cell size and contraction-frequency responses in rat cardiac myocytes. *J Exp Biol* 204: 1191–1199, 2001.
35. **Natali AJ, Wilson LA, Peckham M, Turner DL, Harrison SM, White E.** Different regional effects of voluntary exercise on the mechanical and electrical properties of rat ventricular myocytes. *J Physiol* 541: 863–875, 2002. doi:[10.1113/jphysiol.2001.013415](https://doi.org/10.1113/jphysiol.2001.013415).
36. **Naylor M, Vasan RS.** Preventing heart failure: the role of physical activity. *Curr Opin Cardiol* 30: 543–550, 2015. doi:[10.1097/HCO.0000000000000206](https://doi.org/10.1097/HCO.0000000000000206).
37. **Nichols CG.** K_{ATP} channels as molecular sensors of cellular metabolism. *Nature* 440: 470–476, 2006. doi:[10.1038/nature04711](https://doi.org/10.1038/nature04711).

38. **Palmer BM, Thayer AM, Snyder SM, Moore RL.** Shortening and [Ca²⁺] dynamics of left ventricular myocytes isolated from exercise-trained rats. *J Appl Physiol (1985)* 85: 2159–2168, 1998.
39. **Paredes RM, Etzler JC, Watts LT, Zheng W, Lechleiter JD.** Chemical calcium indicators. *Methods* 46: 143–151, 2008. doi:[10.1016/j.ymeth.2008.09.025](https://doi.org/10.1016/j.ymeth.2008.09.025).
40. **Stones R, Billeter R, Zhang H, Harrison S, White E.** The role of transient outward K⁺ current in electrical remodelling induced by voluntary exercise in female rat hearts. *Basic Res Cardiol* 104: 643–652, 2009. doi:[10.1007/s00395-009-0030-6](https://doi.org/10.1007/s00395-009-0030-6).
41. **Stones R, Natali A, Billeter R, Harrison S, White E.** Voluntary exercise-induced changes in f32-adrenoceptor signalling in rat ventricular myocytes. *Exp Physiol* 93: 1065–1075, 2008. doi:[10.1113/expphysiol.2008.042598](https://doi.org/10.1113/expphysiol.2008.042598).
42. **Sun ZQ, Ojamaa K, Coetzee WA, Artman M, Klein I.** Effects of thyroid hormone on action potential and repolarizing currents in rat ventricular myocytes. *Am J Physiol Endocrinol Metab* 278: E302–E307, 2000.
43. **Taggart P, Sutton PM, Opthof T, Coronel R, Trimlett R, Pugsley W, Kallis P.** Inhomogeneous transmural conduction during early ischaemia in patients with coronary artery disease. *J Mol Cell Cardiol* 32: 621–630, 2000. doi:[10.1006/jmcc.2000.1105](https://doi.org/10.1006/jmcc.2000.1105).
44. **Terrenoire C, Clancy CE, Cormier JW, Sampson KJ, Kass RS.** Autonomic control of cardiac action potentials: role of potassium channel kinetics in response to sympathetic stimulation. *Circ Res* 96: e25–e34, 2005. doi:[10.1161/01.RES.0000160555.58046.9a](https://doi.org/10.1161/01.RES.0000160555.58046.9a).
45. **Thomas D, Kiehn J, Katus HA, Karle CA.** Adrenergic regulation of the rapid component of the cardiac delayed rectifier potassium current, *I_{Kr}*, and the underlying hERG ion channel. *Basic Res Cardiol* 99: 279–287, 2004. doi:[10.1007/s00395-004-0474-7](https://doi.org/10.1007/s00395-004-0474-7).
46. **van der Heyden MA, Wijnhoven TJ, Opthof T.** Molecular aspects of adrenergic modulation of the transient outward current. *Cardiovasc Res* 71: 430–442, 2006. doi:[10.1016/j.cardiores.2006.04.012](https://doi.org/10.1016/j.cardiores.2006.04.012).
47. **Watts VL, Sepulveda FM, Cingolani OH, Ho AS, Niu X, Kim R, Miller KL, Vandegaer K, Bedja D, Gabrielson KL, Rameau G, O'Rourke B, Kass DA, Barouch LA.** Anti-hypertrophic and anti-oxidant effect of f33-adrenergic stimulation in myocytes requires differential neuronal NOS phosphorylation. *J Mol Cell Cardiol* 62: 8–17, 2013. doi:[10.1016/j.yjmcc.2013.04.025](https://doi.org/10.1016/j.yjmcc.2013.04.025).
48. **Winder WW, Hagberg JM, Hickson RC, Ehsani AA, McLane JA.** Time course of sympathoadrenal adaptation to endurance exercise training in man. *J Appl Physiol Respir Environ Exerc Physiol* 45: 370–374, 1978.
49. **Wisløff U, Loennechen JP, Currie S, Smith GL, Ellingsen Ø.** Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca²⁺ sensitivity and SERCA-2 in rat after myocardial infarction. *Cardio-vasc Res* 54: 162–174, 2002. doi:[10.1016/S0008-6363\(01\)00565-X](https://doi.org/10.1016/S0008-6363(01)00565-X).
50. **Wisløff U, Støylen A, Loennechen JP, Bruvold M, Rognum Ø, Haram PM, Tjønnå AE, Helgerud J, Slørdahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen Ø, Skjaerpe T.** Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* 115: 3086–3094, 2007. doi:[10.1161/CIRCULATIONAHA.106.675041](https://doi.org/10.1161/CIRCULATIONAHA.106.675041).
51. **Wisløff U, Loennechen JP, Falck G, Beisvag V, Currie S, Smith G, Ellingsen O.** Increased contractility and calcium sensitivity in cardiac myocytes isolated from endurance trained rats. *Cardiovasc Res* 50: 495–508, 2001. doi:[10.1016/S0008-6363\(01\)00210-3](https://doi.org/10.1016/S0008-6363(01)00210-3).
52. **Woo AY, Xiao RP.** f3-Adrenergic receptor subtype signaling in heart: from bench to bedside. *Acta Pharmacol Sin* 33: 335–341, 2012. doi:[10.1038/aps.2011.201](https://doi.org/10.1038/aps.2011.201).
53. **Woodiwiss AJ, Norton GR.** Exercise-induced cardiac hypertrophy is associated with an increased myocardial compliance. *J Appl Physiol (1985)* 78: 1303–1311, 1995.
54. **Woodiwiss AJ, Oosthuysen T, Norton GR.** Reduced cardiac stiffness following exercise is associated with preserved myocardial collagen characteristics in the rat. *Eur J Appl Physiol Occup Physiol* 78: 148–154, 1998. doi:[10.1007/s004210050400](https://doi.org/10.1007/s004210050400).
55. **Workman AJ, Marshall GE, Rankin AC, Smith GL, Dempster J.** Transient outward K⁺ current reduction prolongs action potentials and promotes afterdepolarisations: a dynamic-clamp study in human and rabbit cardiac atrial myocytes. *J Physiol* 590: 4289–4305, 2012. doi:[10.1113/jphysiol.2012.235986](https://doi.org/10.1113/jphysiol.2012.235986).

56. **Zhu Y, Ai X, Oster RA, Bers DM, Pogwizd SM.** Sex differences in repolarization and slow delayed rectifier potassium current and their regulation by sympathetic stimulation in rabbits. *Pflügers Arch* 465: 805–818, 2013. doi:[10.1007/s00424-012-1193-9](https://doi.org/10.1007/s00424-012-1193-9).
57. **Zingman LV, Zhu Z, Sierra A, Stepniak E, Burnett CM, Maksymov G, Anderson ME, Coetzee WA, Hodgson-Zingman DM.** Exercise- induced expression of cardiac ATP-sensitive potassium channels promotes action potential shortening and energy conservation. *J Mol Cell Cardiol* 51: 72–81, 2011. doi:[10.1016/j.yjmcc.2011.03.010](https://doi.org/10.1016/j.yjmcc.2011.03.010).