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The role of the intrinsic parasympathetic nervous system on training-induced changes of automatism, conduction and refractoriness. An experimental study.

Zarzoso M[1], Such-Miquel L[2], Parra G[3], Brines-Ferrando L[1], Such L[1], Chorro FJ[4], Guerrero J[5], Guill A[6], O'Connor E[7], Alberola A[1].

[1] Department of Physiology, University of Valencia.
[2] Department of Physiotherapy, University of Valencia.
[3] Department of Physical Education and Sports, University of Valencia.
[4] Department of Medicine, University of Valencia and INCLIVA.
[5] Department of Electronic Engineering, University of Valencia.
[6] Department of Electronic Engineering, Polytechnic University of Valencia.
[7] Department of Biochemistry, University of Valencia.

Abstract

The purpose of this study is to test the role that the intrinsic parasympathetic nervous system could play on the adaptive electrophysiological changes produced by physical training on intrinsic myocardial automatism, conduction and refractoriness. Trained rabbits were submitted to a physical training protocol on a treadmill for six weeks. The electrophysiological study was performed on an isolated heart preparation. The investigated myocardial properties were: a) Sinusal automatism; b) Atrioventricular and ventriculoatrial conduction; c) Atrial, conduction system and ventricular refractoriness. The parameters to study the refractoriness were obtained by means of an extrastimulus test at four different pacing cycle lengths (10% shorter than spontaneous sinus cycle length, 250, 200 and 150 ms; and d) Mean dominant frequency (DF) of the induced ventricular fibrillation (VF), using a spectral method. The electrophysiological protocol was performed before and during continuous atropine administration (1μM), in order to block cholinergic receptors. Cholinergic receptor blockade did not modify either the increase in sinusal cicle length, atrioventricular conduction and refractoriness (left ventricular and atrioventricular conduction system functional refractory periods) or the decrease of DF of VF. These findings reveal that the myocardial electrophysiological modifications produced by physical training are not mediated by intrinsic cardiac parasympathetic activity.

Key words: physical training, intrinsic parasympathetic nervous system, heart electrophysiology.

Running title: Parasympathetic activity and training-induced myocardial adaptations.

Introduction

The effects of aerobic endurance training on sinus chronotropism and atrioventricular conduction are well known. Indeed, a decrease in the resting heart rate and a depressed atrioventricular conduction has been reported in human and experimental studies (19, 5, 36). It has been proposed that these modifications are the result of an increased resting vagal tone (5, 33, 34). However, data presented by several investigations in which sympathetic and parasympathetic blockade was performed after an exercise training period have shown a decrease in the intrinsic heart rate (20, 19, 27, 6, 37) and an intrinsic atrioventricular conduction depression (37). Furthermore, similar results have been obtained in experimental models using isolated heart preparations not only on sinus chronotropism (28, 38, 39) and atrioventricular node conduction (38), but also on ventricular refractoriness, which increased by training (38, 39).

On the other hand= However (do you mean that or Moreover/ In addition), the heart contains postganglionic cholinergic neurons, as it is well known, distributed throughout each major atrial and ventricular intracardiac ganglionated plexus (30, 31, 3, 18). It has been reported that these intracardiac neurons display ongoing activity (11) even after acute decentralization (1, 2) and can modify cardiac activity (18, 15), although their precise function and physiological relevance is not completely known (4).

Since the involvement of parasympathetic postganglionic neurons on automatism, conduction and ventricular refractoriness modifications produced by chronic exercise is not well-known, our purpose is to investigate the role of intrinsic cholinergic neurons on these electrophysiological modifications in a model of isolated heart preparation from trained rabbits. We hypothesize that the electrophysiological changes produced by physical training are mediated by parasympathetic neurons contained in the intracardiac ganglia.

Materials and methods

Animals and study design

Thirty-two male New Zealand white rabbits (O*ryctolagus cuniculus*) were used in the present study. Animals were divided into three experimental groups: a trained group (n=11), a control group (n=11) and a sham-operated group (n=10). Animals in the control and sham operated groups were housed in the animal quarters for 46 days and rabbits in the trained group were submitted to a physical exercise program. After familiarization with treadmill running for 4 days, animals in the trained group ran 5 days/week for 6 weeks at 0.33 m/s. Each training session was divided into six periods of 4 minutes of running and 1 minute of rest (39). The correct execution of treadmill exercise was constantly supervised and those animals that did not adequately run on the treadmill, because they either stopped frequently or ran irregularly, were excluded from the study. Housing conditions and experimental procedures used in this study were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23; Revised 1985) and with the approval from the Institutional Animal Care and Use Committee.

Preparation and perfusion

Following heparinization and anesthesia (ketamine 25 $mg \cdot kg^{-1}$, i.v.), animals were euthanized. After thoracotomy, the heart was quickly removed and immersed in cold (4ºC) Tyrode solution for further preparation. The aorta was cannulated and connected to a Langendorff system to provide the heart with warmed, oxygenated Tyrode solution containing (in mM) 130 NaCl, 5.6 KCl, 2.2 $CaCl_2$, 0.6 $MgCl_2$, 1.4 NaH_2PO_4, 25 $NaHCO_3$, and 12.2 glucose. Oxygenation was carried out with a mixture of 95% O_2 and 5% CO_2. Tyrode temperature was constant throughout the experiment (37 ± 0.5ºC), and perfusion pressure was maintained at 60 mmHg.

Two bipolar surface electrodes (silver wire, Teflon-coated) with an inter-electrode distance of 1 mm were positioned on the right atrium for recording and pacing, and an identical electrode was placed on the left ventricle for pacing. Ventricular recordings were made by means of a plaque with 240 unipolar stainless steel electrodes (electrode diameter = 0.125 mm, interelectrode distance = 1 mm) positioned at the epicardial surface of the lateral wall of the left ventricle. The indifferent electrode was a 4 x 6 mm stainless steel plaque located over the cannulated aorta. Recordings were obtained with a cardiac electrical activity mapping

system (MAPTECH, Waalre, the Netherlands). The electrograms were amplified with a gain of 100–300, broad-band (1–400 Hz) filtered and multiplexed. The sampling rate in each channel was 1 kHz. Electrical stimuli were delivered by a Grass S-88 stimulator (Grass Instruments, Quincy, MA, USA) connected to a stimulus isolation unit.

Measurements and calculations

In the experiments, we used the same procedure with trained and non-trained rabbits. The parameters studied and their definitions were the following: 1) Sinus cycle length: the interval between two successive ventricular electrograms during basal sinus rhythm (V–V interval); 2) A-V interval: the interval between an atrial electrogram and its corresponding ventricular electrogram during basal sinus rhythm; 3) Wenckebach cycle length (WCL): the maximum cycle length of atrial pacing that produces A-V Wenckebach block; 4) Retrograde WCL (RWCL): the maximum cycle length of ventricular pacing that impedes a 1:1 V-A conduction; 5) Atrial effective refractory period (AERP): the maximum atrial extrastimulus coupling interval without atrial capture; 6) Atrial functional refractory period (AFRP): the minimum interval between the atrial electrogram produced by the last basic atrial train stimulus and that was triggered by the extrastimulus; (do you mean the passive as I corrected it?) 7) Ventricular effective refractory period (VERP): the maximum ventricular extrastimulus coupling interval without ventricular capture; 8) Ventricular functional refractory period (VFRP): the minimum interval between the ventricle electrogram produced by the last basic ventricular train stimulus and that was triggered by the extrastimulus; 9) A-V conduction system effective refractory period (AVCSERP): the maximum atrial electrogram coupling interval produced by the extrastimulus without ventricular capture; 10) A-V conduction system functional refractory period (AVCSFRP): the minimum interval between the ventricular electrogram produced by the last basic atrial train stimulus and that was triggered by the extrastimulus; 11) Effective refractory period of the V-A retrograde conduction system (VACSERP): the maximum ventricular electrogram coupling interval, produced by the extrastimulus, without atrial capture; 12) Functional refractory period of the V-A retrograde conduction system (VACSFRP): the minimum interval between the atrial electrogram produced by the last basic ventricular train stimulus and that was triggered by the ventricular extrastimulus; 13) Mean dominant frequency (DF) of ventricular fibrillation (VF): the frequency of the power spectrum with the greatest amplitude, analyzed in consecutive segments of 4 seconds using Welch's method.

In order to investigate these parameters, we performed several tests according to the following protocol: 1) Atrial pacing at increasing frequencies (2 $Hz \cdot s^{-1}$) to calculate WCL; 2) Atrial extrastimulus testing using basic trains of 10 stimuli at four different pacing cycle lengths: 10% shorter than sinus cycle length and of 250, 200 and 150 ms. The pause between trains was 1 second and the extrastimulus was delivered with 5 ms decrements in each of the trains of stimuli starting from the coupling interval 10% lower than the sinus cycle length, 250, 200 and 150 ms. This test was used to determine atrial and A-V conduction refractoriness; 3) Ventricular extrastimulus testing, using the same technique as in atrial extrastimulus testing, was performed to evaluate ventricular and V-A retrograde conduction refractoriness; 4) Ventricular pacing at increasing frequencies (2 $Hz.s^{-1}$) to obtain RWCL and induce VF; 5) Recording of ventricular electrograms during VF (330 s). Coronary perfusion was maintained during the arrhythmia. After 10 minutes of stabilization, 1µM atropine sulphate (Sigma-Aldrich, St. Louis, MO, USA), dissolved in Tyrode, was administered through the aorta in continuous infusion. Maintaining the infusion of atropine, we repeated the electrophysiological protocol to evaluate any possible changes derived from parasympathetic activity in the parameters studied after muscarinic receptors blockade. In the sham-operated group, the same volume of filtered Tyrode without atropine was infused because we wanted to confirm if the course of time or another manoeuvre such as the repetition of stimulation protocol could modify the results. V–V interval was measured after the initial period of stabilization, immediately before and after atropine and/or Tyrode infusion.

Stimuli of 2 ms duration and twice diastolic threshold were used in the stimulation protocol. Mean atrial diastolic threshold was 8.8±5 mA(is it written like this??), while the mean ventricular diastolic threshold reached values of 14.2±5 mA.

Coronary flow was weighed after collection for 1 min. Measurements were performed before starting the electrophysiological protocol, before and after the infusion of atropine, and also at the end of the protocol. Each heart was weighed after the experiment had finished.

Data analysis

All data are expressed as mean ± SD. Comparisons of refractory periods and DF of VF were made using a two-way ANOVA with repeated measures. Paired and unpaired Student's t-test were used to compare R-R

interval, A-V interval, WCL and RWCL, coronary flow and heart weights between or within groups when necessary. Statistical significance was accepted when $P < 0.05$.

Results

Sinusal chronotropism

In the sham-operated group, the course of time or the experimental protocol repetition did not modify V-V interval (Table 1). Likewise, cholinergic blockade did not modify V-V interval in the control and trained groups. Basal sinus cycle length was 16% longer in the trained versus the control group.

Table 1. Sinusal chronotropism

Group	Basal	Pre-infusion	Post-infusion
	V-V	V-V	V-V
Control	305±34 (10)	347±40 (9)	350±38 (9)
Trained	354±34* (11)	391±31 (7)	394±24 (7)
Sham	314±35 (10)	328±47 (9)	327±46 (9)

Values are means ± SD in ms. Number of experiments in parentheses. V-V = ventricular depolarization to ventricular depolarization; A-V = A-V interval. *P<0.05 vs control.

A-V and V-A conduction

After atropine administration, no differences in A-V interval and WCL were obtained within the control and trained groups (Table 2). WCL was 10% longer in the trained group with respect to the control group. In the sham-operated and control groups, RWCL underwent a 4% and 8% increase after Tyrode and atropine infusion, respectively.

Table 2. Parameters of A-V and V-A conduction

	Pre-infusion			Post-infusion		
	A-V	WCL	RWCL	A-V	WCL	RWCL
Control	67±6 (9)	125±12 (9)	165±18 (9)	67±7 (9)	137±13 (9)	178±18† (9)
Trained	66±3 (7)	137±12* (11)	176±22 (10)	66±7 (7)	148±28 (11)	179±21 (10)
Sham	67±6 (9)	125±10 (7)	178±20 (8)	67±7 (9)	135±22 (7)	190±20† (8)

Values are means ± SD in ms. Number of experiments in parentheses. A-V = A-V interval; WCL = Wenckebach cycle length; RWCL = retrograde Wenckebach cycle length; *P<0.05 vs control; †P<0.05 vs pre-infusion.

Atrial, ventricular, A-V nodal, and V-A retrograde conduction system refractoriness

Neither the effective nor the functional atrial, ventricular, A-V and V-A conduction system refractory periods were modified by the infusion of atropine (Figure 1A-F). The functional refractory period of the left ventricle was longer in trained animals vs. controls at the four pacing cycle lengths: 156 vs 138 ms at a coupling interval 10% lower than the sinus cycle length, 144 vs 129 ms at 250 ms pacing cycle length, 133 vs 118 ms at 200 ms pacing cycle length and 122 vs 110 ms at 150 ms pacing cycle length (Figure 1D). Similarly, a larger A-V conduction system functional refractory period was observed in the trained group: 178 vs 152 ms at a coupling interval 10% lower than the sinus cycle length, 160 vs 145 ms at 250 ms pacing cycle length and 151 vs 140 ms at at 200 ms pacing cycle length (Figure 1E). No significant differences were found in the remaining parameters of refractoriness between trained and untrained animals. AVCSERP and VACSERP were not analyzed because, in most cases, the atrial or ventricular effective refractory period was reached before the conduction system refractory period took place. Results obtained in the sham-operated group indicate that none of these parameters of refractoriness were modified by the course of time or the experimental protocol repetition (Tables 3, 4 and 5).

Table 3. Parameters of atrial refractoriness

	AERP				AFRP			
	10% SCL	250 ms	200 ms	150 ms	10% SCL	250 ms	200 ms	150 ms
Pre-infusion	87±12 (6)	88±7 (6)	89±9 (6)	81±8 (6)	106±8 (6)	106±9 (6)	105±10 (6)	99±5 (6)
Post-infusion	87±9 (6)	84±8 (6)	82±11 (6)	81±10 (6)	102±3 (6)	100±5 (6)	96±4 (6)	95±3 (6)

Values are means ± SD in ms. Number of experiments in parentheses. AERP = atrial effective refractory period; AFRP = atrial functional refractory period; 10% SCL = pacing frequency 10% shorter than spontaneous sinus cycle length; 250, 200 and 150 = pacing cycle length of 150, 200 and 150 ms.

Table 4. Parameters of ventricular refractoriness

	VERP				VFRP			
	10% SCL	250 ms	200 ms	150 ms	10% SCL	250 ms	200 ms	150 ms
Pre-infusion	118±20 (5)	104±14 (5)	97±11 (5)	83±13 (5)	137±19 (5)	123±11 (5)	115±10 (5)	105±8 (5)
Post-infusion	123±23 (5)	112±16 (5)	101±18 (5)	98±20 (5)	138±19 (5)	124±12 (5)	112±15 (5)	107±16 (5)

Values are means ± SD in ms. Number of experiments in parentheses. VERP = ventricular effective refractory period; VFRP = ventricular functional refractory period; 10% SCL = pacing frequency 10% shorter than spontaneous sinus cycle length; 250, 200 and 150 = pacing cycle length of 150, 200 and 150 ms.

Table 5. Parameters of conduction system refractoriness

	AVCSFRP				VACSFRP		
	10% SCL	250 ms	200 ms	150 ms	10% SCL	250 ms	200 ms
Pre-infusion	155±8 (7)	147±8 (7)	139±3 (7)	130±6 (7)	169±16 (5)	158±9 (5)	153±11 (5)
Post-infusion	153±9 (7)	141±6 (7)	134±6 (7)	128±6 (7)	166±14 (5)	156±7 (5)	153±12 (5)

Values are means ± SD in ms. Number of experiments in parentheses. AVCSFRP = atrioventricular conduction system effective refractory period; VACSFRP = ventricleatrial conduction system functional refractory period; 10% SCL = pacing frequency 10% shorter than spontaneous sinus cycle length; 250, 200 and 150 = pacing cycle length of 150, 200 and 150 ms.

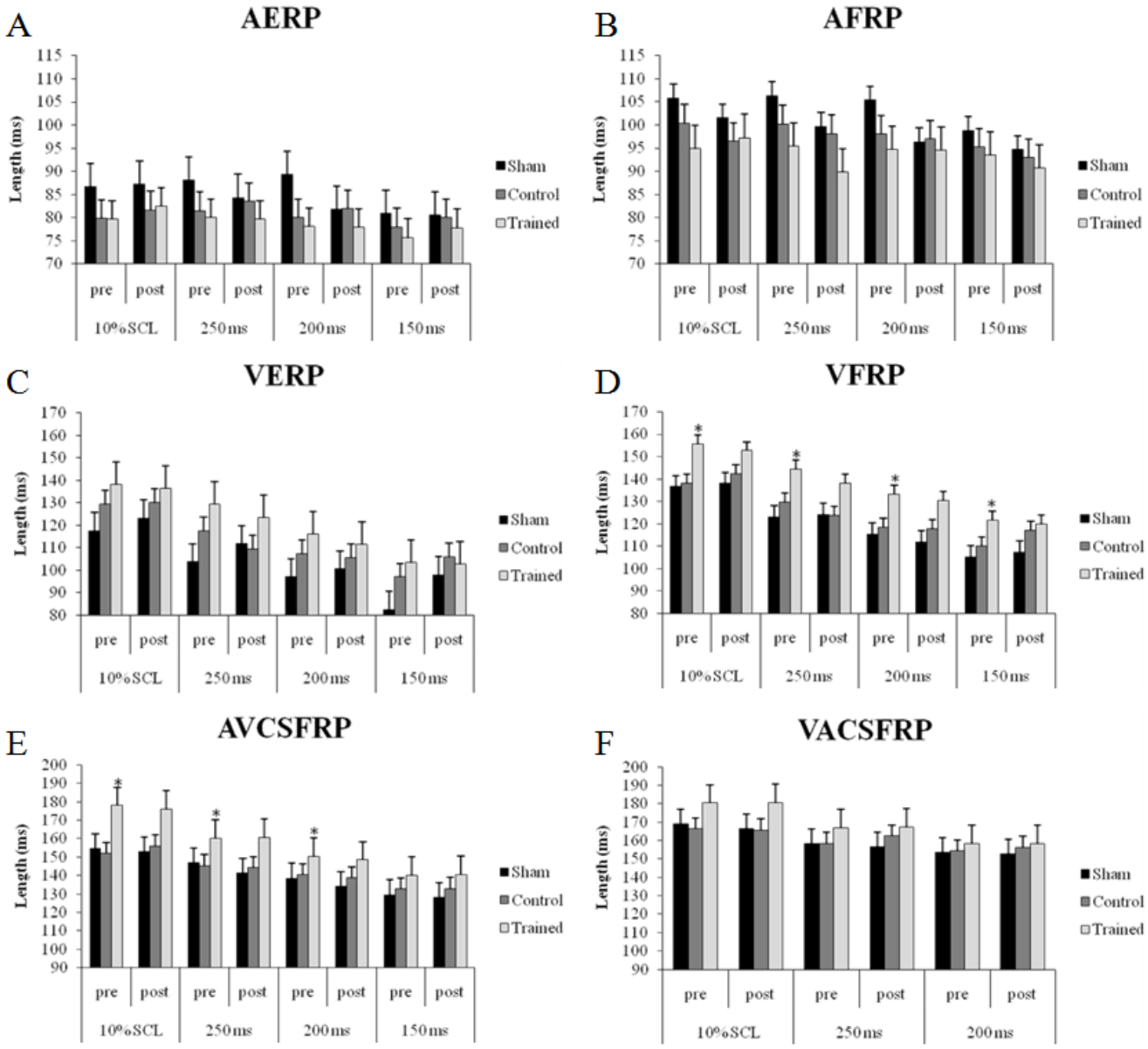

Figure 1. Myocardial refractory periods before (pre) and after atropine/Tyrode infusion (post). No differences were obtained after parasympathetic blockade in AERP (A), AFRP (B), VERP (C), VFRP (D), AVCSFRP (E) and VACSFRP (F). 10% SCL = pacing frequency 10% shorter than spontaneous sinus cycle length; 250, 200 and 150 = pacing cycle length of 150, 200 and 150 ms; *P<0.05 vs control. Error bars display the standard error of the mean.

Dominant frequency of ventricular fibrillation.

There were no differences in the DF of VF in the sham-operated group when comparisons before and after Tyrode infusion were made. In this group, DF of VF reached values of 22.0 ± 3.0 Hz immediately after VF triggering and decreased to 15.6 ± 3.3 Hz at 300 s. After Tyrode infusion, DF decreased from 24.3 ± 6.2 to 17.4 ± 4.6 Hz. Similar kinetics were observed in the trained and control groups and no changes were found after cholinergic blockade on the mean DF of VF of both groups (Figures 2A and 2B). The mean DF of VF was lower in the trained group (Figure 2C).

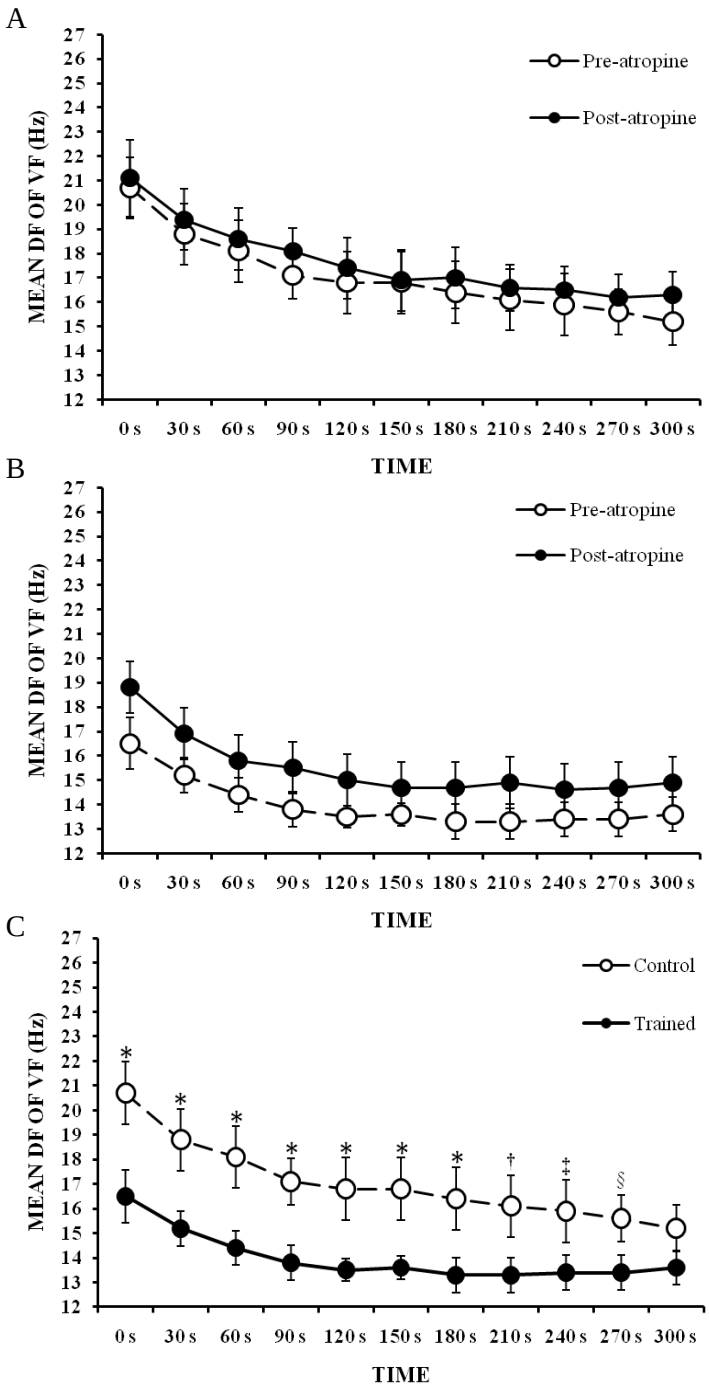

Figure 2. Dominant frequency of ventricular fibrillation. Parasympathetic blockade did not modify this parameter in the control (A) and trained (B) groups. Furthermore, DF of VF was lower in the trained group (C) *P<0.05 vs control; †P = 0.08 ; ‡P = 0.06; §P = 0,09. Error bars display the standard error of the mean.

Coronary flow and heart weights

Coronary flow was not different between the trained and control groups when expressed in milliliters per minute per gram (3.24 ± 0.9 in the trained group vs 3.34 ± 0.9 $ml \cdot min^{-1} \cdot g^{-1}$ in the control group). Likewise, parasympathetic blockade did not modify coronary flow within the control and trained groups. In the sham-operated group, the course of time or the experimental protocol repetition did not modify this parameter. Heart weights were similar in trained and control rabbits (14.4 ± 2 and 14.2 ± 2 g, respectively).

Discussion

We have studied the role of the intrinsic parasympathetic nervous system on training-induced modifications of the myocardial electrophysiological properties: automatism, conduction and refractoriness. The main findings of this study are that the parasympathetic blockade did not modify the effect of physical training on both sinus node automatism and atrioventricular conduction, and atrioventricular conduction system and ventricular refractoriness.

Methodological considerations

It must be noted that the experimental preparation used is not a "working heart" and the differences observed between groups do not result from differences in cardiac work (39). Moreover, it has been reported that rabbits provide a good experimental model for the investigation of cardiac electrophysiology. As it is known the electrophysiological properties and VF characteristics in the rabbit are more similar to that observed in humans than rats or mice (29, 12), which have major limitations in performing cardiac electrophysiological studies (13). Finally, by using the proper intensity, duration and frequency of exercise, the rabbit obtains a documented cardiovascular training effect rather easily (10). Indeed, it has been shown that the training protocol used reduces heart rate *in vivo* and *in vitro* (39), which is considered to be one of the most fundamental systemic characteristics of the trained state (26). Moreover, the training protocol produced myocardial HSP60 and iNOs expression modifications, (39) which are associated with physical training (16, 14).

Effect of parasympathetic blockade on automatism and conduction

As we have shown in the Results section, physical training depressed sinus node automatism and A-V nodal conduction. These results are partially in accordance with those previously obtained in human (20, 19, 6, 36, 37) and experimental studies (28, 27, 38, 39). Nevertheless, muscarinic receptor blockade with atropine did not modify the negative chronotropic and dromotropic effects produced by physical training. It has been reported that the increase in resting cardiac parasympathetic activity produced by physical training underlies the decrease in resting heart rate and the depression of A-V conduction. As it is well known, the release of acetylcholine (ACh) from parasympathetic postganglionic terminals causes a muscarinic receptor-mediated opening of specific potassium channels (I_{KACh}) and other effects on several pacemaker currents that

result in slowing the firing rate of pacemaker cells and a delay in AV conduction (8, 35). As the intrinsic cardiac nervous system displays ongoing activity even when it is disconnected from the higher nervous centers (1, 2) and can modify cardiac function (18, 15), the changes observed by previous studies using a similar experimental model (28, 38, 39) could have been mediated by intrinsic nervous activity. In view of the present results, intrinsic parasympathetic nervous system activity does not explain the bradycardia and A-V conduction delay in isolated hearts from trained animals. Additionally, we did obtain differences in RWCL in the sham and control groups, an increase being produced after the infusion of Tyrode/atropine, respectively. These results are quite surprising, as parasympathetic blockade should have enhanced impulse conduction and decreased RWCL, given that ACh depresses conduction. Hence, the increase in RWCL does not seem to be an atropine related effect.

Refractoriness and parasympathetic blockade

With respect to the effects of cholinergic blockade, the changes produced by physical training on myocardial refractoriness were not mediated by intrinsic parasympathetic activity, as we did not find modifications by atropine infusion in ventricular or atrioventricular conduction system refractoriness.

The study of ventricular refractoriness was carried out using two different methods: ventricular extrastimulus testing and spectral analysis of VF. Both measures provide a reliable method to assess ventricular refractoriness, not only with a common method used in electrophysiological studies (extrastimulus test) but also with the DF of VF. This last parameter expresses the speed of ventricular activation during the VF and inversely correlates with VFRP, thus being and indirect measure of ventricular refractoriness (9). Moreover, this procedure let us analyze up to 240 different points of ventricular myocardium in each experiment during VF.

Our results show that the increase in ventricular refractoriness produced by physical training, assessed by the increase in VFRP and the decrease in DF of VF, was not altered after parasympathetic blockade. With respect to the increase of ventricular refractoriness, these results are similar to those obtained in previous studies (39). The prolongation of ventricular refractoriness could be related to intrinsic parasympathetic activity since parasympathetic postganglionic neurons are also found in the ventricle and their activity can lead to a release of ACh even in the isolated heart (22). This is consistent with experimental studies which

have reported a prolonging effect of vagal nerve stimulation on ventricular refractoriness (23, 17), even without background sympathetic activity (21). Nevertheless, atropine administration did not modify the training-induced increase in ventricular refractoriness, indicating that this cardiac electrophysiological modification is not mediated by the activation of I_{KACh}.

As far as A-V conduction system refractoriness is concerned, we found that physical training increased AVCSFRP and this increase was not abolished after atropine infusion. Consequently, ACh does not seem to be implicated in the A-V conduction system refractoriness increase produced by physical training. These results are consistent with Stein et al. (37), which found that atrioventricular node effective refractory period in athletes was longer than in untrained individuals even after pharmacological blockade, indicating that these changes were caused by intrinsic electrophysiological modifications. Neither physical training nor parasympathetic blockade modified retrograde conduction system refractoriness.

With respect to atrial refractoriness and its modification by physical training, it has been reported either no change (7) or an increase (24) in athletes with Wolff-Parkinson-White syndrome. Experimental studies carried out in isolated rabbit heart have shown that although the differences were not statistically significant, AERP tended to increase ($p = 0.09$) in the hearts of trained animals (39). In the present study, physical training did not change AERP and AFRP and these parameters were not modified after parasympathetic blockade. Regarding the effect of parasympathetic stimulation, acetylcholine release from parasympathetic postganglionic terminals in the atria activates I_{KACh}, which leads to a shortening of the AERP, a decrease in the action potential duration and an increase in the dispersion of refractoriness (41, 40). These electrophysiological modifications facilitate the induction and maintenance of atrial fibrillation, whose incidence has been reported to be higher in long-term endurance athletes (25). Data presented indicate that physical training does not modify atrial refractoriness and that there is no ACh release from parasympathetic postganglionic terminals in atrial intracardiac ganglia to exert any influence in atrial refractoriness in the isolated rabbit heart model.

The results obtained not only in the intrinsic heart rate and atrioventricular conduction but also in myocardial ventricular and atrioventricular node refractoriness, suggest a contributing role of an intrinsic adaptation induced by physical training. The observed electrophysiological modifications are exhibited in an isolated heart preparation and thus not submitted to extrinsic nervous system and/or humoral influences.

Regarding the basic mechanisms implied in these modifications, we have discarded a functional implication of I_{KACh} by means of muscarinic blockade with atropine, which implies that these intrinsic modifications are not dependent on intrinsic parasympathetic activity. Previous studies carried out by our research team reported that these changes do not seem to be related to heart hypertrophy, lipid peroxidation, and/or coronary flow modifications either (38).

In conclusion, our findings reveal that the myocardial electrophysiological modifications produced by physical training are not mediated by intrinsic cardiac parasympathetic activity. As parasympathetic blockade did not modify the electrophysiological properties studied in isolated rabbit heart, other intrinsic modifications must be implied.

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