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ELECTROCARDIOGRAPHIC VENTRICULAR REPOLARISATION PROCESSES IN ANDALUSIAN HORSES BEFORE AND AFTER PHYSICAL TRAINING

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Summary

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Equine ventricular repolarisation wave can be influenced by many physiological and pathological factors. T wave abnormalities have been related to a shorter time for ventricular filling, reduced stroke volume, cardiac output and exercise performance in racehorses. The present research performed electrocardiographic recordings in 14 four-year-old male Andalusian horses, when they were untrained and after three months of an aerobic training programme. Leads I, aVF, V10, V1R, V3R, V1L and V3L were used. It was aimed to assess the incidence of abnormal T waves in this breed, according to the criteria of abnormalities established for other equine breeds, to evaluate if the changes induced by training in the T wave are the same that those reported in the athletic heart syndrome in canine and human athletes and, to analyse if the abnormal T waves could have been related to changes in the plasma concentrations of Na, K, and Cl, since these electrolytes are involved in the cardiac electrical processes.

It was found out that the incidence of abnormal T waves was quite high in the Andalusian breed, especially in the precordial leads. Moreover, the incidence of abnormal T waves increased in precordial leads and decreased in lead I and aVF after training. The abnormal T waves after training were shorter and had the same voltage, changes which were different to those presented for the athlete' heart syndrome. The horses with abnormal T waves had higher plasma K concentrations, both before and after training. Plasma Na and Cl concentration at rest decreased after training. Plasma Na concentrations were positively related to T wave duration and negatively to T wave voltage.

Key words: electrocardiography, heart, horses, performance, training

INTRODUCTION

The equine ventricular repolarisation wave has received a great interest as early as 1957, when Brooijmans reported the changes linked to exercise and concluded that elevation or depression of T waves to values exceeding the normal should be regarded as evidence of coronary insufficiency. However, the T wave is the most variable waveform in the electrocardiogram and may be influenced by a great number of factors. For that reason, quantification of its diagnostic significance can be difficult. Thus, the T wave alterations have been associated with several systemic physiological and pathological conditions, including exertion, excitement, drugs, infectious diseases, vagal tone, blood electrolyte imbalances, hypoxia, myocarditis, and myocardiosis (Stewart *et al.*, 1983; Evans, 1991). It is important to consider that the T wave alterations induced by exercise and excitation are very similar to those described in myocarditis and infectious diseases (Persson & Forssberg, 1987), which makes its clinical evaluation more difficult.

It is accepted that both humans and dogs that participate in regular vigorous or strenuous physical activities undergo significant changes in cardiac structure and function (Bjornstad et al., 1993; Constable et al., 1994; Pellecia et al., 2000). The morphological changes consist of increases in left-ventricular end-diastolic cavity dimension, maximum wall thickness, mass index and left atrial dimension (Peleccia et al., 2000). These adaptations related to training significantly alter the ventricular depolarisation and repolarisation waves, although the most common changes are those of early repolarisation (Huston et al., 1985). The alterations in the T wave can occur in one of the two ways: tall and peaked, or inverted. Tall, peaked T waves are frequently seen as part of the early repolarisation syndrome. Furthermore, increased amplitude is detected, which may reflect repolarisation changes or may be merely secondary to repolarisation of increased ventricular mass (Turpeinen et al., 1996).

It is not clear if these modifications appear also in the equine electrocardiogram, since the horse belongs to the category B of Hamlin & Smith (1965), characterised by only two general fronts of ventricular depolarisation instead of the three described for the species of category A (man, monkey, cat and rat). In the horse, the major masses of both ventricles are excited with a single burst of depolarisation, due to the more general penetration of the Purkinje fibres into the epicardium. Since this activation occurs without spread in any particular direction, as polarity reversals exist within the free walls of the ventricles, the contribution to the genesis of the depolarisation complex of the electrocardiogram recorded at the body surface is quite little (Hamlin & Smith, 1965). However, it must be taken into account that the sequences of ventricular activation and repolarisation clearly differ, since the T wave in the normal heart does not have an equal area and opposite polarity to the QRS complex (Muñoz *et al.*, 1995a; Muñoz *et al.*, 1995b).

On the other hand, a retrospective study concluded that some T-wave abnormalities could have been associated with poor performance. Moreover, these animals presented a slower recovery of the heart rate after exercise (Rose et al., 1980). During exercise, horses with abnormal resting electrocardiograms may have a shorter time for ventricular filling and a reduced stroke volume and cardiac output (Stewart et al., 1983). Similarly, the horses with abnormal ventricular repolarisation waves seem to have reduced exercise tolerance, with low values of LAo' and LA9 (plasma lactate concentrations immediately after exercise and at 9 m/s, respectively). These characteristics have been related to a state of overtraining, with red cell hypervolaemia, excessive heart rate and lactate responses to exercise in relation to red cell volume and, probably, a dystonia of the autonomic nervous system (Persson & Forssberg, 1987).

In the present study, the morphology, the voltage and the duration of the ventricular repolarisation wave are analysed before and after three-month of training in Andalusian horses. Three are the main objectives: 1) To assess the incidence of abnormal T waves in Andalusian horses, according to the criteria of abnormalities established for other equine breeds, *i.e.* Thoroughbred racehorses and Standardbred trotters, 2) To evaluate if the changes induced by the training in this wave are the same that those reported in the athletic heart syndrome in dogs and human athletes and 3) To analyse if the horses with abnormal T waves have different resting plasma concentrations of the main electrolytes involved in cardiac electrical processes.

MATERIALS AND METHODS

Horses

Sixteen four-year-old Andalusian (AN) horses was included in the present research. All of them were male and belonged to the same Horse Training Centre. Before starting the study, the animals were subjected to a cardiovascular examination, consisting of heart and respiratory auscultations, both at rest and after a slight exercise at trotting, and routine haematology and plasma biochemistry. Only the animals without any abnormality were studied. At the beginning of the research, the horses were untrained.

Electrocardiograph recordings

Electrocardiograms were recorded at rest in standing position. A portable singlechannel electrocardiograph (Cardiostat-T, Siemens) was used, at a chart velocity of 25 mm/s and a sensitivity of 10 mm/mV. The electrodes were attached to skin folds by alligator clips and electrocardiographic gel. After that, a variable period of time was waited, according to the degree of excitation of the animals, in order to avoid the emotional tachycardia and the influence of the duration of diastole on the pattern of ventricular repolarisation. For

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that reason, only electrocardiograms with heart rates lower than 45 beats/min were measured. No tranquillisers were administered along the electrocardiographic study and care was taken to ensure that the limbs were correctly positioned.

Electrocardiographic leads

A total of seven leads were recorded: I, aVF, V10, V1R, V3R, V1L, and V3L. The leads I, aVF and V10 were the semiorthogonal system, which allowed to record the electrical activity in the heart as projections on three different body planes, i.e., frontal (Lead I), horizontal (aVF) and sagittal (V10) (Hamlin & Smith, 1965). The other four were precordial leads. The electrodes were placed as follows: a) at both elbow joints, the positive electrode on the right forelimb (I); b) at the left stifle joint (aVF); c) at the spinal apophysis of the 7th thoracic vertebra (V10); d) on the right hemithorax, roughly 5 cm dorsal to the ventral midline, and approximately 5 cm caudal to the olecranon (V1R); e) at the cranial tip of the right shoulder joint (V3R); f) on the left hemithorax, roughly 5 cm dorsal to the ventral midline, and approximately 5 cm caudal to the olecranon (V1L) and g) at the cranial tip of the left shoulder joint (V3L).

Training programme

The horses underwent two electrographical controls, when they were untrained and after three months of a predominantly aerobic training programme. The training exercises involved a daily schedule of 20–30 min of walking, 25–30 min of trotting and 12–18 min of galloping on a sandy track. The daily routine was carried out 5 days per week: 5–7 min of trotting, walking-trotting (as a transition to galloping), 5–9 min of galloping, trotting and finally, a few minutes of galloping. The intensity of this programme was quite low, since its function was to improve physical fitness to prepare the animal for breeding purposes. Despite its low intensity, it has been shown that this programme enhanced the aerobic capacity, since the glycolytic response to exercise with lactate formation was significantly reduced after training. Furthermore, the anaerobic threshold increased (Muñoz *et al.*, 1999b).

Electrocardiographic measurements

The percentage of the different morphologies of the T wave was calculated for each lead, after measuring 10 cardiac cycles. T waves were classified as simples or doubles, the former being positive (P) or negative (N) and the latter, biphid (with the two components of the same polarity, P/P or N/N) or biphasic (with the two components of different polarity, P/N or N/P). The duration and the voltage of the T wave and the heart rate (HR) were measured and were expressed as the mean of the 10 cardiac cycles. In the double T waves, the voltage was measured separately for those waves with a single deflection and those with two deflections (T1 and T2).

Electrocardiographic criteria of abnormality

The criteria of electrocardiographic abnormal depolarisation waves described for racehorses were followed (Stewart *et al.*, 1983; Evans, 1991). T waves were considered abnormal if they were positive or biphasic in lead I, negative in aVR, and positive in the precordial leads V1R, V1L, V3R and V3L. The V10 lead has not been considered in the criteria of abnormalities, since the exploratory electrode was located dorsally to the heart. The presence of three or more abnormal T waves in the 10 cardiac cycle measured, was used as evidence of abnormality. According to these criteria, horses were classified as having abnormal (AB) or normal (NM) T waves.

Electrolyte determinations

Immediately after finishing the electrocardiographic recordings, jugular blood samples were withdrawn and poured into tubes with lithium heparin as anticoagulant. The samples were centrifuged during the first 5 minutes after collection, and the supernatant was harvested. All the plasma samples were kept refrigerated at 4-8°C until analysis, performed within the first 12 hours after collection. Plasma sodium (Na), potassium (K) and chloride (Cl) concentrations were measured with an automatic analyser with selective electrodes for ions (Ciba Corning 644).

Statistical study

To compare the incidence of T wave abnormalities in three or more leads in the untrained and trained groups, a chi-square test was carried out. A Student's test was used to assess the changes in HR, duration and voltage of the T waves and plasma electrolyte concentrations. Finally, in order to investigate the possible effect of HR and plasma ion levels on T wave characteristics, a linear correlation analysis (Pearson linear correlation) was performed. All the data are presented as means \pm standard deviations (SD). Significant differences were considered at p<0.05.

RESULTS

A great variation in the T wave morphology was observed in all the leads, although I, V1R and V1L seemed to be the most homogeneous. The predominant morphologies were N in leads I and V10, P in V1R and V1L, N/P in aVF and P/N

| | | Ι | aVF | V10 | V1R | V1L | V3R | V3L |
|--------------|----------------------|--------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| P (+) | Untrained | | 24.17 | 38.19 | 66.67 | 77.78 | 37.50 | 4.17 |
| | Trained | | 33.33 | 43.75 | 83.33* | 92.31* | 48.13 | 15.38* |
| N (-) | Untrained Trained | 91.67 100 | 11.11 | 41.67 31.25 | | | 8.333 10.69 | 16.67 37.95* |
| P/N (+/-) | Untrained Trained | | 14.72 6.333 | 20.14 16.67 | 25.00 16.67 | | 45.84 41.18 | 79.16 46.67* |
| N/P (-/+) | Untrained Trained | 8.333 | 50.00 60.34 | 8.333 | 8.333 | 22.22 7.69* | 8.333 | |

Table 1. Percentages of the different T wave morphologies in the seven electrocardiographic leads in untrained and trained Andalusian horses

* P<0.05 related to training.

Table 2. Percentages of abnormal (AB) and normal (NM) T wave morphologies in untrained and trained Andalusian horses in I, aVF and precordial leads

| | | Lead I | Lead aVF | Precordial leads |
|--------------------------------|-----------|--------|-------------|------------------|
| Number of horses with AB waves | Untrained | 3 | 2 | 6 |
| | Trained | 1 | 0 | 8 |
| Number of horses with NM waves | Untrained | 13 | 14 | 10 |
| | Trained | 15 | 16 | 8 |
| Incidence of abnormalities (%) | Untrained | 18.75 | 12.50 | 37.50 |
| | Trained | 6.25* | 0* | 50.00* |

* P<0.05 related to training.

in V3R and V3L. The percentages of the different morphologies in the seven studied leads before and after training are summarised in Table 1. No P/P or N/N configurations were found in any lead (Table 1). The incidence of AB-T and NM-T waves is reported in Table 2. The training programme induced a decrease in the incidence of abnormalities of T waves in leads I and aVF, and an increase in the four precordial leads.

The differences in HR, T wave electrocardiographic features and resting plasma electrolyte concentrations between AB and NM horses in the two controls (untrained, trained) are presented in Fig. 1, 2 and 3. No differences in HR between AB and NM horses were found, neither before nor after training (Fig. 1). However, training caused a significantly resting bradychardia in both groups.

The duration of the T waves ranged between 0.105 and 0.175 s in the AB group and between 0.125 and 0.185 s in the NM group (Fig. 2). No differences in T wave duration were observed between AB and NM horses before training. Trained AB horses had significantly shorter T waves than trained NM horses. Training induced a reduction in the duration of the ventricular repolarisation wave in both groups of horses.



Fig. 1. Heart rate in Andalusian horses with abnormal and normal T wave morphology, before (white bars) and after (grey bars) training. The data are presented as mean \pm SD; n=16. Level of significance – a: training effect in horses with abnormal T waves at P<0.05; n: training effect in horses with normal T waves at P<0.05.

The voltage of the T wave (Fig. 2) ranged between 0.250 and 0.710 mV in the AB group and between 0.250 and 0.580 mV in the NM group. AB horses showed higher voltage than NM horses before training. Training induced a reduction in T wave voltage in AB and NM horses.

Fig. 3 presents the plasma concentrations of the three studied electrolytes. Resting plasma K concentrations were similar in AB and NM horses before training. On the other hand, AB horses presented significantly higher plasma K concentrations than NM horses after training. Furthermore, training caused a significant reduction in plasma Na and Cl concentrations in both AB and NM horses.

The results of the correlation analysis are presented in Table 3. HR was negatively correlated with the voltage of the T wave, but no significant correlation was detected with the duration. In relation to the plasma electrolyte concentrations, the



Fig. 2. T wave duration (left) and T wave voltage (right) in Andalusian horses with abnormal and normal T wave morphology, before (white bars) and after (grey bars) training. The data are presented as mean \pm SD; n=16. Level of significance – a: training effect in horses with abnormal T waves at P<0.05; n: training effect in horses with normal T waves at P<0.05; * P<0.05 between AB and NM horses before training; ** P<0.05 between AB and NM horses after training.



Fig. 3. Plasma electrolyte concentrations in Andalusian horses with abnormal and normal T wave morphology, before (white bars) and after (grey bars) training. The data are presented as mean \pm SD; n=16. Level of significance – a: training effect in horses with abnormal T waves at P<0.05; n: training effect in horses with normal T waves at P<0.05; * P<0.05 between AB and NM horses before training; ** P<0.05 between AB and NM horses after training.

| | Lead I | | Lead aVF | | Precordial leads | |
|----------|---------|----------|----------|----------|------------------|----------|
| | Voltage | Duration | Voltage | Duration | Voltage | Duration |
| HR | -0.710* | 0.480 | -0.810* | 0.240 | -0.840* | 0.240 |
| Na | -0.670* | 0.720* | -0.690* | 0.720* | -0.740* | 0.840* |
| Κ | 0.310 | -0.160 | 0.210 | -0.180 | 0.090 | -0.200 |
| Cl | -0.210 | 0.250 | 0.250 | 0.250 | -0.500 | 0.520 |
| * D<0.05 | | | | | | |

Table 3. Correlation coefficients between electrocardiographic characteristics of T wave, heart rate (HR) and plasma Na, K and Cl concentrations in leads I, aVF and precordials

* P<0.05.

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strongest correlations were found between electrocardiographic features and plasma Na. Plasma Na levels concentrations were positively correlated with T wave duration and inversely with T wave voltage. The other two electrolytes did not influence the duration and the voltage of the T wave.

DISCUSSION

The present research focused on the electrocardiographic features of the ventricular repolarisation wave in AN horses before and after training. A high percentage of morphologically AB T waves, both in untrained and trained groups, together with bradycardia, reduced duration and voltage and decreased plasma Na and Cl concentrations after training were the most marked results.

One striking finding was the high percentage of AB T wave according to the criteria of abnormalities established for other equine breeds. Four main reason could be considered in order to explain this high incidence: influence of HR, limb position during electrocardiographic recordings, shape of the thorax and location of the heart in the AN breed and, effect of the plasma K concentrations.

Changes in T wave are often thought to be due to neurohormonal regulatory influences, even if great care is taken to avoid emotional stress and tachycardia. Persson & Fossberg (1987) defended the hypothesis of the neurodystonic T wave changes since they found that AB T waves are normalised very rapidly. However, HR of the AN horses in the present research was similar to that described for Thoroughbred racehorses and Standardbred trotters (Stewart *et al.*, 1983; Evans *et al.*, 2002). Furthermore, special care was taken when recording, and only those electrocardiograms with a HR lower than 45 beats/min were considered. Moreover, the incidence of AB T waves increased after training in spite of the reduction in HR.

It has been suggested that improper placement of the electrodes in the limbs, as well as their position could strongly influence the morphology of the electrocardiographic waves (Muñoz *et al.*, 1995a). For that reason, the position of the limbs and electrodes were carefully controlled. However, it must be noted that the percentage of AB T waves was higher in the precordial leads, where electrodes are not placed in the limbs.

There is not available literature about the morphometric characteristics of the AN breed, and therefore, the effect of the depth of the thorax on the electrocardiographic recordings could not be analysed. According to the authors, AN breed has a deeper thorax than Thoroughbred and Standardbred trotters, but the influence of this peculiarity on the T wave morphology is unknown.

Finally, it should be considered that the horses with AB T waves presented higher plasma K concentrations. A decreased K extracellular concentration causes a prolongation of the action potential by lengthening of phase 3, which could result in a delay in the repolarisation time of the myocardium and as a consequence, the ST interval increased and the T waves flattened (Escabias et al., 1990). In our study, the opposite fact was observed, since the horses with higher plasma K concentrations showed shorter T waves. Moreover, the voltage was reduced after training without any change in the kalemia. Escabias et al. (1990) found that the hyperkalemia in AN foals was associated with T waves of a smaller amplitude, instead of the expected peaked and tall

waves. These authors related the results to the age of the animals, since only neonatal AN foals were studied. However, according to the data of the present research, the influence of the breed should also be taken into consideration.

The AB T waves before training had similar duration as the NM T waves. By contrast, after training, AB T waves were flatter. These adaptations differed from those described for the athlete' heart syndrome, with tall, peaked T waves, related to a training-induced decrease in sympathetic tone, which uncovers an inherent asymmetry of repolarisation (Zeppelli *et al.*, 1980; Spataro *et al.*, 1998).

It is recognised in dogs and humans that an increase in cardiac mass will induce greater duration of the waves, since the duration of the electrocardiographic processes represents the time necessary for the electrical fronts to travel along the myocardium (Huston et al., 1985; Bjornstad et al., 1993; Constable et al., 1994). Surprisingly, in the present research, a reduced duration of T wave was found in the AB horses after training. Similar results have been already described in this equine breed (Muñoz et al., 1995a ; Muñoz et al., 1995b). It has been proposed that the cardiac hypertrophy linked to the training could generate a greater distribution of Purkinje fibres, and therefore, the velocity of the electrical processes should be greater and the electrocardiographic waves shorter. The same results have been found in the present research.

The T wave voltage decreased after training in both AB and NM groups. Three main hypotheses have been proposed for this finding. Firstly, a greater development of the cardiac conduction system would increase the number of cancelled dipoles. Secondly, the recordings of electrocardiographical voltage on the body surface are affected by the orientation of the myocardial fibres and the effective dipoles of the activation waves are parallel to the longitudinal axes of these fibres. The eccentric and circumferential growth of the left ventricular wall and the degree of dilatation of the ventricular cavity due to aerobic training could modify the orientation of the myocardial fibres (Stadler et al., 1993; Young, 1999). Thirdly, the short-circuit effect exerted by blood volume on the longitudinal components of the electrical forces could reduce the voltage of the T wave, the opposite of it was expected according to the Brody effect. This effect explains that an increase in volume of a cardiac cavity rises the electrical forces of the walls of that cavity and therefore, the wave voltage in the electrocardiogram (Vancheri & Barber, 1989). It cannot be affirmed that these AN horses were highly trained and although a left ventricular hypertrophy could have occurred, this study could not demonstrate a relationship between size of the ventricular wall and T wave characteristics. In spite of this, the effect of the HR must be considered, since HR was negatively correlated with T wave voltage. More studies, including echocardiograms and histopathological studies would be necessary to elucidate these hypotheses.

Some previous studies have shown that poorly performing racehorses have a significantly higher incidence of electrocardiographic abnormalities when comparing with those presented for routine examinations or muscle-skeletal diseases (Stewart *et al.*, 1983; Persson & Forssberg, 1987). Along the period of study, the same AN horses were subjected to different exercise tolerance tests (Muñoz *et al.*, 1999b; Muñoz *et al.*, 1999c) and muscle biopsies (Data not published) were taken from the gluteus medius to deterElectrocardiographic ventricular repolarisation processes in Andalusian horses ...

mine enzyme activities. Decreases in HR and plasma lactate responses in relation to work intensity are regarded as an effect of training, indicating an improvement in aerobic potential and fitness (Davie et al., 2002; Muñoz et al., 2002). It can be affirmed that our AN horses showed an enhancement of fitness, with higher aerobic and anaerobic thresholds. Furthermore, muscle activities of citrate synthase (CS) and 3-OH-acyl Coenzyme A dehydrogenase (HAD), representing the aerobic power of the Krebs cycle and the lipid βoxidation rose after training. By contrast, the capacities of breakdown glycogen and to reduce pyruvate to lactate were reduced, as demonstrated by the glycogen phosphorylase (PHOS) and lactate dehydrogenase activities (LDH). It is thought that better performers have higher oxidative potential (Muñoz et al., 1999a). In spite of the improved physical fitness of the studied AN horses, the percentage of AB T waves increased.

The electrocardiogram has been considered a sensitive indicator of changes in plasma concentrations of Ca and K and a poor indicator of those of Na and H (Escabias et al., 1990). In AN foals, the time taken by the excitation wave to spread through the atrioventricular node, bundle of His and its branches (PQ segment) and the electrical ventricular systole duration (QT interval) were strongly influenced by plasma concentrations of Na. Consequently, a slight decrease of Na, even within the normal range, may cause an acceleration in the conduction of the excitation. In our research, on the other hand, the plasma Na concentration was correlated with the voltage and the duration of the ventricular repolarisation wave. However, the direction of the correlation with the duration was the inverse of that found

by Escabias *et al.* (1990), result that may be due to the different age of the animals.

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