COMPUTERIZED AND DYNAMIC ELECTROCARDIOGRAPHIC EVALUATION IN DOGS WITH ACUTE NORMOVOLEMIC ANEMIA

AVALIAÇÃO ELETROCARDIOGRÁFICA COMPUTADORIZADA E DINÂMICA EM CÃES COM ANEMIA NORMOVOLÊMICA AGUDA

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SUMMARY
The cardiovascular response to acute normovolemic anemia is related to activation of compensatory mechanisms that can cause changes in the electrocardiogram. The electrocardiographic examination demonstrates the occurrence of arrhythmias and may suggest the presence of atrial and ventricular enlargement or myocardium hypoxia. The aim of this study is to describe cardiac rhythm, heart rate and ECG pattern in dogs undergoing acute normovolemic anemia. The dogs were submitted to computerized electrocardiography 15 minutes and 24 hours after induction of anemia and evaluated by Holter, with 24 hours of continuous electrocardiographic monitoring. Anemic dogs had no arrhythmias or premature ventricular and atrial complexes related to hypoxia and it were observed reductions in the incidence of sinus arrhythmia. Although there was no development of sinus tachycardia, there was a significant increase (P = 0.01) in heart rate (bpm) in both assessments made after installation of anemia (100.90 ± 23.1 to 132.50 ± 12.7 and 132.70 ± 23.9). The increased heart rate was linearly related to the fall in hematocrit (R² = 0.27, P = 0.018). In addition, this study identified changes in the amplitude and duration of P wave and PR interval, without clinical significance because they were within the normal range for the specie. Therefore, dogs with acute normovolemic anemia and volemic reposition with Ringer’s lactate solution and hydroxyethyl starch-based, with hematocrit of 14.37 ± 2.99%, did not develop significantly arrhythmias, extrasystoles or electrocardiographic patterns consistent with myocardial hypoxia and the electrocardiographic changes found may be related compensatory mechanisms activated.


RESUMO
A resposta cardiovascular à anemia normovolêmica aguda está relacionada à ativação de mecanismos compensatórios que podem acarretar alterações ao eletrocardiograma. O exame eletrocardiográfico demonstra a ocorrência de arritmias, bem como pode sugerir a presença de sobrecarga atrial e ventricular ou hipóxia do miocárdio. O objetivo deste estudo é descrever o ritmo cardíaco, frequência cardíaca e padrão eletrocardiográfico em cães submetidos à anemia normovolêmica aguda. Foram realizados registros eletrocardiográficos computadorizados e eletrocardiografia dinâmica com holter de 24 horas. Os cães anêmicos não apresentaram arritmias ou extrasistoles atriais e ventriculares relacionadas à hipóxia e observou-se diminuição da incidência de arritmia sinusal. Apesar de não haver desenvolvimento de taquicardia sinusal, houve aumento significativo (P=0,01) da frequência cardíaca (bpm) nas duas avaliações realizadas após a instalação da anemia (100,90 ± 23,1 para 132,50 ± 12,7 e 132,70 ± 23,9), relacionando-se ao hematocrito (R² = 0,27; P = 0,018). Foram identificadas alterações na amplitude e duração de onda P e intervalo PR, porém sem significado clínico pois estavam dentro dos valores de normalidade para a espécie. Portanto, cães com anemia normovolêmica aguda e reposição volêmica com solução Ringer com lactato e amido hidroxietílico, com hematocrito de 14,37 ± 2,99%, não desenvolveram arritmias ou alterações eletrocardiográficas compatíveis com hipóxia do miocárdio significativas, apresentaram aumento da frequência cardíaca linearmente relacionado à queda do hematocrito e as alterações encontradas podem ter relação com os mecanismos compensatórios ativados.


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INTRODUCTION

Acute normovolemic anemia results from the withdrawal of blood and simultaneous replacement with the appropriate volume of colloid and crystalloid fluids to maintain blood volume (JAMNICKI et al., 2003). This procedure has been used as a model of anemia in dogs (KOMA et al., 2005, SPOTSWOOD et al., 2005, KOMA et al., 2006) and to reduce the requirement for blood transfusions during surgical procedures, since it can improve the oxygen supply to tissues (HABLER et al., 1996, LICCKER et al., 2007).

In anesthetized patients, reducing the oxygen-carrying capacity during acute normovolemic hemodilution is offset by increases in cardiac output and stroke volume. Therefore, the oxygen supply to organs remains unchanged with hematocrit levels of 20-25% (HABLER et al., 1996, JAMNICKI et al., 2003, LICCKER et al., 2007). However, there are hemoglobin levels in which oxygen consumption is not maintained by compensatory mechanisms (JAMNICKI, 2003).

Various electrocardiographic abnormalities have been described in humans with anemia (STANOJEVIC & STANKOV, 1998, KIKUCHI & INAGAKI, 1999, BAILEY ET AL., 2003). According to Stanoevic e Stankov (1998), the incidence of electrocardiographic changes in anemic humans is 10 to 80% and varies with the severity of anemia and some abnormalities may occur only after the stress test.

Major electrocardiographic abnormalities related to anemia are abnormalities in ST segment, T wave and QT interval (STANOJEVIC & STANKOV, 1998; LEUNG et al., 2000, BAILEY et al., 2003). However, Aessopos et al. (2001) described electrocardiographic changes in anemic humans suggestive of left and right ventricular hypertrophy, right axis deviation, right bundle branch block, third degree atrioventricular block, premature atrial contraction and atrial fibrillation. In dogs and cats, some electrocardiographic changes associated with anemia were described, such as sinus tachycardia (BAHL et al. 1992, TILLEY, 1992) and premature ventricular complexes (TILLEY, 1992).

In situations of acute normovolemic anemia, Scheller et al. (2010) observed a gradual prolongation of the QT and the QTc-interval, reduction of the amplitude of the T-wave and a QRS-complex partly diminished in amplitude in pigs and the authors discuss a possible involvement of myocardial hypoxia.

The aim of this study was to describe the cardiac rhythm, heart rate and electrocardiographic pattern in conscious dogs submitted to acute normovolemic anemia and its correlation with low levels of hematocrit.

MATERIALS AND METHODS

The study group comprised ten healthy adult mongrel dogs, seven males and three females, with mean weight of 14.81 ± 1.90 kg. Before the study, blood count, serum chemistry profile (creatinine, alanine aminotransferase, total protein and albumin), electrocardiography, Doppler echocardiography and systemic blood pressure measurement were performed.

This study was in accordance with ethical principles in animal experimentation adopted by the Brazilian College of Animal Experimentation and was reviewed and approved by the Ethics and Animal Welfare committee of Sao Paulo State University (protocol number 018344-07).

Induction of acute normovolemic anemia followed the model described by Koma et al. (2005) and Spotswood et al. (2005), with objectives to provide low hematocrit values with minimum risks for the dog. For the induction of anemia, approximately 20% of total circulating blood was removed by jugular venipuncture every 12-24 hours for 3-4 days to obtain a hematocrit below 18%. The total circulating blood volume of each animal was calculated as eight percent of body weight. Simultaneously with the withdrawal of blood, the animal received the same volume of Ringer's lactate solution and hydroxethyl starch-based solution in a 2:1 ratio to maintain normovolemia.

Blood samples for laboratory tests were collected by jugular venipuncture and placed in vials containing ethilenediaminetetraacetic acid (EDTA). The counts of eritrocytes and hemoglobin determinations were performed in automatic cell count device².

The animals underwent daily physical examination and electrocardiographic monitoring was performed in two forms, by computerized electrocardiography³ realized 15 minutes (T1) and 24 hours (T2) after induction of anemia and by dynamic electrocardiography, using a 24 hour Holter monitor continuously during this period. After assessment at 24 hours, all dogs received autologous blood transfusion to normalize erythrocyte values.

The computerized electrocardiography was performed in right lateral recumbence using a 6-lead computerized electrocardiogram and the electrode clips were attached to the animal’s skin in the standard positions as described by Tilley (1995). The paper speed was 50mm/sec and the electrocardiogram was standardized at 1mV = 1cm. The electrocardiogram analysis was obtained from lead II, where the parameters duration of P wave (Pms), P wave amplitude (PmV), duration of PR interval (PRms), duration of QRS complex (QRSms), R wave amplitude (RmV), duration of QT interval (QTms), ST segment amplitude (ST), T wave amplitude (T), mean electrical axis, heart rate (HR) and heart rhythm were evaluated.

The dynamic electrocardiographic monitoring was performed using a Holter electrocardiogram device in two pre-cordial leads (rV2 and V4) during the period of 24 hours. The electrodes were placed on the shaved chest and the device packaged in a leather vest, allowing the free movement of the dog. During the 24 hours, the dogs were housed in kennels with restricted access and any procedures performed at this site, such as handling the dog, cleaning the kennels, water and

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² ACT-8 Coulter Miami - EUA
food supply, were noted in order to rule out influences of the external environment in this examination.

The data obtained in the experiment were statistically analyzed by the computer program ProcGLM Statistical Analysis System (SAS®), using analysis of variance (ANOVA), followed by Tukey's test to compare the means of groups over time and linear regression was used to access the relation of hematocrit with the heart rate, with the level of significance at 5%.

**RESULTS**

The basal values ± standard deviation of erythrocytes was 6248 ± 821.92 x 10⁶/mm³, hemoglobin was 13.92 ± 1.81g/dl and hematocrit was 42.7 ± 5.75%. After normovolemic anemia, the respective values of erythrocytes, hemoglobin and hematocrit were 2037 ± 352.77 x 10⁶/mm³, 4.59 ± 0.87g/dl and 14.37 ± 2.99%.

In assessing cardiac rhythm, all dogs showed respiratory sinus arrhythmia at baseline, with 40% of wandering pacemaker. In the evaluation after 24 hours of installation of anemia, 70% of dogs presented respiratory sinus arrhythmia with 40% of wandering pacemaker and 30% had sinus rhythm. The mean baseline value of heart rate was 100.90 ± 23.1 bpm and after acute normovolemic anemia, it was observed increases (P = 0.01) in both evaluations T1 (132.50 ± 12.7 bpm) and T2 (132.50 ± 12.7 bpm). Linear regression revealed a relationship between low hematocrit and heart rate (R² = 0.27, P = 0.018) and the corresponding regression equation is heart rate = 141 – 0.852 hematocrit. Besides increasing of heart rate, among the electrocardiographic parameters examined, only the variables P-wave duration, P wave amplitude and PR interval showed statistically significant variations (P<0.05). The average values of P-wave duration in dogs evaluated at T2 differed from T1, but both showed no statistically significant difference compared to baseline (T0). The P wave amplitude, in turn, has decreased compared to baseline, though not statistically different from the average of evaluations performed in T2. The PR interval in T1 was a statistically lower (P = 0.005) than baseline and T2.

The electrocardiographic variables P wave duration (ms) and P wave amplitude (mV), PR interval (ms), QRS duration (ms), QT interval (ms), R wave amplitude (mV), heart rate (bpm) and mean ventricular axis (°) are statistically analyzed in Table 1.

There were no elevation or depression in ST segment of anemic dogs, however, there was slurring of the ST segment in 20% of dogs in group T1. Finally, 100% of dogs had normal T-wave amplitude (less than 25% of R wave). Regarding the polarity of the T wave, 70% of dogs of T1 group had biphasic waves, 20% had positive waves and 10% negative waves and 50 % of dogs of T2 group had biphasic waves, 40% positive and 10% negative waves, with no polarity reversal at any time during the electrocardiographic monitoring.

As for dynamic electrocardiography (Holter), no animal had ventricular premature complexes or atrial premature complexes. Additionally, no changes in depression or ST-segment elevation were observed in anemic dogs. The mean and standard deviation of the QRS complexes were 156205.4 ± 25486.27 in 24 hours of anemic status. The mean values ± standard deviation of maximum, mean and minimum heart rate were respectively 204 ± 29.66 bpm, 111.6 ± 17.23 bpm and 63 ± 11.77 bpm. The average number of pauses longer than 1.5 seconds was 42.2 ± 70.91 and the longer pauses had mean duration of 2.06 ± 0.56 seconds.

**DISCUSSION**

In the computerized electrocardiographic evaluation in dogs of this study, there were no changes suggestive of hypoxia, myocardial infarction or electrolyte imbalance, nor arrhythmias, except the occurrence of slurring of ST segment in 20% of dogs from T1 group. This corroborates the findings described by Stanoevic and Stankov (1998), which showed absence or low prevalence of arrhythmias or electrocardiographic changes suggestive of hypoxia at rest, showing them only after exercise or stress testing.

| Table 1 - Mean values ± standard deviations of electrocardiographic parameters of dogs (n=10), before (basal), 15 minutes (T1) e 24 hours (T2) after induction of acute normovolemic anemia. |

<table>
<thead>
<tr>
<th>Parametes</th>
<th>Basal</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(ms)</td>
<td>42.5 ± 3.3⁴</td>
<td>40.60 ± 4.8⁹</td>
<td>44.90 ± 5.6⁹</td>
</tr>
<tr>
<td>P(mV)</td>
<td>0.19 ± 0.1⁴</td>
<td>0.15 ± 0.04⁶</td>
<td>0.16 ± 0.04⁹</td>
</tr>
<tr>
<td>PR(ms)</td>
<td>99.60 ± 10.7⁴</td>
<td>87.80 ± 9.0⁹</td>
<td>97.00 ± 12.1²</td>
</tr>
<tr>
<td>QRS(ms)</td>
<td>58.40 ± 6.9⁴</td>
<td>55.40 ± 4.6⁶</td>
<td>58.00 ± 7.1⁹</td>
</tr>
<tr>
<td>QT(ms)</td>
<td>210.10 ± 18.8⁴</td>
<td>201.00 ± 10.2⁴</td>
<td>193.7 ± 13.8⁹</td>
</tr>
<tr>
<td>R (mV)</td>
<td>1.24 ± 0.5⁵</td>
<td>1.10 ± 0.3³</td>
<td>1.15 ± 0.3³</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>100.90 ± 23.1³</td>
<td>132.50 ± 12.7³</td>
<td>132.70 ± 23.9³</td>
</tr>
<tr>
<td>Axis(°)</td>
<td>75.90 ± 13.1³</td>
<td>66.5 ± 26.4⁶</td>
<td>75.5 ± 10.6⁶</td>
</tr>
</tbody>
</table>

Letters indicate significant differences (p < 0.05). P (ms) = P wave duration; P(mV) = P wave amplitude; PR (ms) = PR interval duration; QRS (ms) = QRS complex duration; QT (ms) = QT interval duration; R (mV) = R wave amplitude; HR (bpm) = Heart rate; Axis(°) = Mean ventricular axis.
In a study realized in anemic humans, electrocardiographic changes were described in 64% of patients with chronic anemia, and at rest, only 3% of these patients demonstrated ST-segment depression while after the stress test occurred ST segment depression in 33%, T-wave inversion in 10% and prolongation of the QT interval by 27% of the patients (STANOJEVIC & STANKOV, 1998). In turn, Bailey et al. (2003) reported, in a human patient, myocardial infarction with ST-segment elevation attributed to severe anemia. ST changes were also found in patients with anemia (MEHTA et al., 1983) and during severe acute isovolemic hemodilution (Leung et al., 2000).

Despite the absence of changes in T wave and ST segment, 20% of dogs showed the characteristic of slurring of this segment, which may also be indicative of myocardial hypoxia. This fact agrees partially with electrocardiographic findings suggestive of myocardial infarction or hypoxia in human patients with anemia, such as changes in ST segment and T wave (MEHTA et al. 1983, LEUNG et al. 2000, BAILEY et al. 2003).

The absence of abnormalities in T wave, ST segment and cardiac rhythm does not indicate hypoxia or myocardial ischemia that could cause cardiac arrhythmias. Hypoxia causes uncoupling electromechanical, i.e., mechanical contractile activity is markedly depressed while the electrical activity (action potential) is initially maintained (MC DONALD & MAC LEOD, 1975). With the persistence of hypoxia, there are modifications in calcium influx by inhibition of the slow calcium channels and there are reduced cellular levels of ATP, particularly in situations of acidic pH (BELARDINELLI & LUCHESE, 1979).

The normal cardiac rhythm can assume the absence of myocardial ischemia related to low hematocrit. During ischemia, high extracellular concentration of potassium in the ischemic region depolarizes the cells and consequently reduces the rest potential to a level where the fast sodium channels are partially inactivated. Furthermore, the release of catecholamines in the ischemic region may increase the density of slow channels available for activation by facilitating the induction of action potential of the slow type (WIT & BIGGER, 1975). As the slow action potential depolarization has low speed, spread very slowly (low speed of conduction), stimulating the development of reentry mechanisms and develop arrhythmias (CRANEFIELD et al., 1971).

Concerning the measurements of electrocardiographic parameters, the significant decrease of P wave duration in T1 and its increase in T2 and the decreases of P waves amplitude in T1 and T2 are not clinically relevant, since they are within the range of reference to the canine species (WOLF et al., 2000). The same is valid for the reduction of PR interval, which is not clinically relevant because the values are considered normal, which relates to the increase of heart rate (TILLEY, 1992).

The absence of significant changes in QRS duration and R wave amplitude agrees with the findings of Ishikawa (1976). In turn, animals with bleeding may decrease the duration of the QRS complex and R wave amplitude due to changes in the electrical transmission in the myocardium, caused by blood loss and consequent reduction in cardiac volume (MANOACH et al., 1971). As the study animals maintained normal blood volume, hardly would present these electrocardiographic characteristics.

The increase of duration and amplitude of P wave, duration of R waves and QRS complex are only suggestive of atrial or ventricular enlargement, requiring other cardiac tests to confirm its occurrence (TILLEY, 1992). Therefore, the absence of increases on these electrocardiographic parameters does not rule out atrial or ventricular overload.

The electrocardiographic interpretation of dogs with acute normovolemic anemia revealed significant increases in heart rate values at T1 and T2, as observed in other studies (METIVIER et al. 2000, SPOTSWOOD et al. 2006). However, the heart rate values were within the normal range for dogs and did not characterize the sinus tachycardia reported in anemic dogs (BAHL et al. 1992, TILLEY, 1992). The increase in heart rate in anemic dogs may explain the decrease in QT interval since they are inversely proportional (TILLEY, 1992), even though this was not statistically significant.

Despite the increase in heart rate, this was not enough to cause arrhythmias, once increased heart rate can lead to cardiac arrhythmias due sustained depolarization caused by accumulation of potassium by inhibition of Na/K/ATPase pump (BELARDINELLI & LUCHESE, 1979).

In this study, there were no influence of anesthetic agents on heart rate, the During acute normovolemic anemia, increased heart rate seems to occur only in people awake, while in anesthetized patients, there is change in heart rate only if develops hypovolemia. The autonomic nervous system depression by anesthetics and central vagal stimulation caused by opioids may explain the absence of tachycardia in anesthetized patients (JAMNICKI, 2003).

Despite of the absence of sinus tachycardia, the change of the incidence of sinus arrhythmia to sinus rhythm in anemic dogs can indicate influence of sympathetic nervous system, since sinus arrhythmia is related to parasympathetic influence. The return of the prevalence of sinus arrhythmia in dogs after 24 hours (T2) shows probable adaptation to anemic state, returning the vagal influence, since the degree of respiratory sinus arrhythmia may be used as a noninvasive indicator of the degree of cardiac parasympathetic control (KATONA & JIH, 1975).

Only one dog presented arrhythmia showing second degree atrioventricular block (BAV) Mobitz type 2, with constant PR intervals before the blockade. In this electrocardiogram, the QRS duration was normal, classifying the BAV type A, assuming in this case that the failure to conduct site is located above the bifurcation of the His bundle. Despite the second-degree BAV Mobitz type 2 usually may be associated with type B, this electrocardiographic abnormality can be found in normal dogs without hemodynamic consequences or relation to anemia (TILLEY, 1992).

The absence of changes in ventricular electrical axis agrees with Singh and Sood (1996) that describes
the absence of ventricular axis deviation, despite of cardiomegaly in human patients with anemia. However, some authors describe axis deviation to the right (MANOACH et al., 1971) or left (ROSENTHAL et al., 1971).

Dynamic electrocardiography (Holter) revealed no abnormalities suggestive of hypoxia, such as ST segment depression, arrhythmias or either atrial or ventricular premature complexes, according to computerized electrocardiography. The analysis of heart rate revealed normal values (TILLEY, 1992), discarding the occurrence of sinus tachycardia in dogs during the 24 hours after induction of anemia.

During severe anemia and normovolemia electrocardiographic changes can be detected and further investigations are warranted to elucidate whether these changes indicate myocardial hypoxia (SCHELLER et al., 2010). The absence of arrhythmias, premature beats or electrocardiographic alterations related to myocardial hypoxia in dogs of this study can be explained by increases blood flow and nutrition in microvascular space caused by decreased blood viscosity, which counteract the reduction of oxygen carrying capacity of blood due to hemoglobin reduction (LICKER et al., 2007).

**CONCLUSION**

Increased heart rate in anemic dogs, although not characterized as sinus tachycardia, had a linear relationship with the reduction in hematocrit. The increase of heart rate and the reduced incidence of sinus arrhythmia can be justified by the sympathetic nervous system activation as a compensatory mechanism to acute normovolemic anemia. This procedure can improve microvascular blood flow due hypoviscosity, hindering the development of cardiac arrhythmias or electrocardiographic pattern suggestive of myocardial hypoxia, as observed on computerized and dynamic electrocardiographic evaluation in this study.

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