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SERUM ELECTROLYTES CHANGES IN HEALTHY DOGS SUBMITTED TO DOBUTAMINE STRESS TEST

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SUMMARY: The aim of this study was to investigate hematologic and serum chemistry changes in healthy dogs subjected to conventional protocol of dobutamine stress test. The study was performed in ten healthy German Shepherd dogs. The dobutamine stress test was carried out as three minute stages protocol, from the starting dose of 7.5 µg/kg/min until the maximum dosage of 42.5 µg/kg/min. Blood samples were collected before and after the dobutamine stress test for complete blood count and chemistry analyses. Statistically significant changes of potassium concentration ($p<0.05$), sodium concentration ($p<0.05$) and chloride concentration ($p<0.001$) were registered after the dobutamine stress test. The registered changes could be explained by the dobutamine effects through β1 adrenergic receptors.

Key words: *dobutamine stress test, electrolytes, dog*

INTRODUCTION

Dobutamine is widely utilized as a drug in human pharmacological stress testing, but dobutamine stress test (DST) is still in the investigational phase in canine medicine. Dobutamine is a potent β1 adrenergic agonist with minimal β2 and α adrenergic effects. Studies in normal conscious dogs (Vatner et al., 1974; Hinds and Hawthorne, 1975; Liang and Hood, 1979) have shown that dobutamine augments cardiac output and myocardial contractility, but has little effect on the heart rate (HR) and aortic blood pressure in the presence of an intact baroreceptor reflex (Liang et al., 1981). The primary effect of dobutamine infusion, as a diagnostic tool, constitutes an increase in heart rate and systolic blood pressure which results in the increase in myocardial oxygen demand, similar to that occurring during moderate physical exercise (Wackers, 1993). A high HR seems to be essential for the ability of the stress test to detect cardiac dysfunction. In order to reach high target HR, many DST protocols use high doses of the drug,

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potentially increasing the risk of adverse effects and complications (Lima et a., 2008). However, because of its brief duration of action dobutamine can be used with relative safety (Jewitt et al., 1974; Wackers, 1993).

By choosing the protocol appropriate for a clinical use, we investigated complete blood count (CBC) and serum chemistry changes in healthy German Shepherd dogs subjected to DST. These parameters represent basic circulatory and metabolic parameters and some of them, like electrolytes, are important for cardiac electrical activity. Former researches of DST in dogs have been conducted in certain breeds (Beagle, Doberman) and no information exist about DST in German Shepherd dogs.

MATERIALS AND METHODS

Animals

Ten German Shepherd dogs (8 males and 2 females) were studied; they were one to nine years old. All dogs were without clinical symptoms of cardiovascular or respiratory diseases. The dogs were evaluated by clinical examination including the history of each dog, physical examination, complete blood count and serum chemistry, electrocardiography, 2D and M-mode echocardiography. All examinations were performed with a manual restraint of the animals, without uses of sedation or anesthesia. The experimental protocol was approved by the the Ethical Committee of the Faculty of Veterinary Medicine University of Belgrade (approval number 01-3 / 2008).

Dobutamine Stress Test

The dobutamine stress test was performed as conventional DST by dobutamine administered via a cephalic vein (Dobutamine, Panpharma S.A., France, 250 mg/ 20ml). The dobutamine initial dose of 7.5 μg/kg/min was increased at 3-minute intervals, by 5 μg/kg/min, until a maximum dosage of 42.5 μg/kg/min was achieved, or when one of the following signs was reached: tachycardia over 200/min, severe arrhythmia or uncontrollable excitement of the animal. Before dobutamine administration baseline blood samples were taken for a complete blood count (CBC): red blood cell (RBC), hemoglobin (Hgb), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular Hgb (MCH), mean corpuscular Hgb concentration (MCHC), and serum chemistry analyses: albumin, protein, blood urea nitrogen (BUN), creatinine, creatin kinase (CK), lactate dehydrogenase (LDH), potassium, sodium, chloride, bicarbonate. Right after termination of dobutamine application (<1 minute), blood samples were taken again and same parameters as before DST were evaluated. Blood samples were assayed on automated analyzers Cell Dyn 3700 (Abbott Diagnostics) and Olympus AU400 (Olympus). During DST cardiac echo and continuous lead II electrocardiogram were monitored in order to recognize the peak of DST and terminate dobutamine infusion.

Statistical Analyses

The results were analyzed by statistical package Statistica 8. The data are reported as mean ± SD. All values before and after DST were compared by Student's t-test for dependant samples. The differences were taken to be significant at $p < 0.05$ and $p < 0.001$.

RESULTS

This DST study was carried out in 10 German Shepherd dogs of different age, showing no cardiovascular and respiratory clinical signs. In a clinical examination of the cardiovascular system, physiological heart sound was observed with no heart murmurs and with regular or regularly irregular rhythm. The arterial and venous pulse were normal. On the basis of the clinical examination (CBC, serum chemistry analysis, electrocardiography, echocardiography) all the dogs were considered free of cardiac diseases and other systemic diseases that could influence the cardiovascular system structure and function.

All dogs tolerated the DST well. The following adverse effects were registered during DST: panting, mild excitement, nausea, weakness of short duration. None of these side effects interrupted the experimental protocol.

The CBC changes after DST consisted of red blood cell increase, hematocrit increase, as well as hemoglobin concentration increase. However, differences in the CBC values before and after DST were not significant. CBC values both before and after DST were within the reference values range.

Table 1. Hematological parameters of dogs before and after DST
Tabela 1. Vrednosti hematoloških parametara kod pasa pre i posle DST

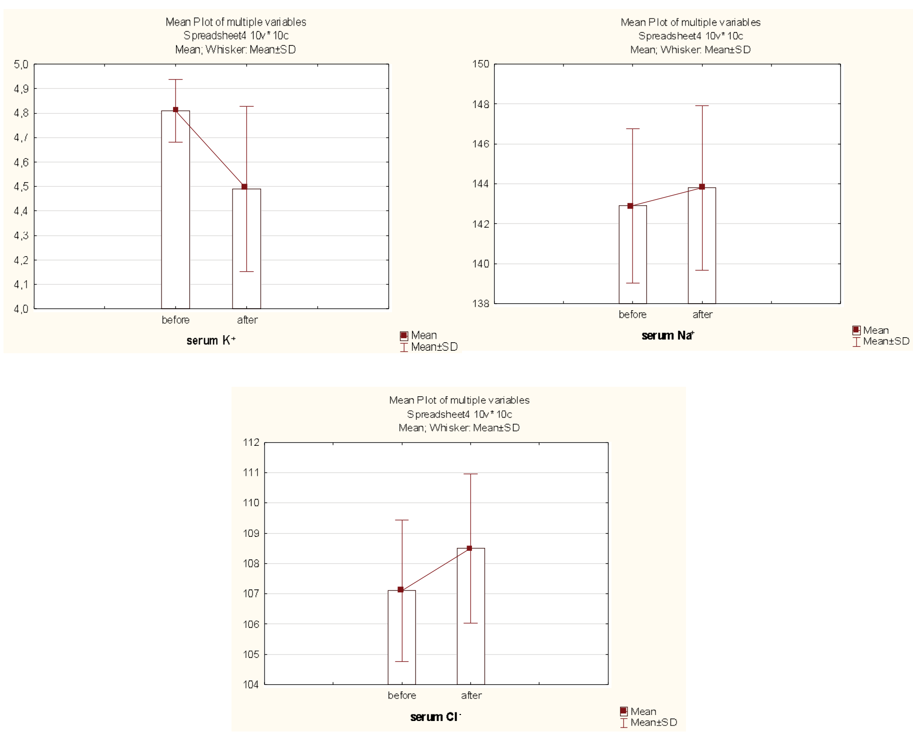
DST	Red blood cell/ *Eritrociti* ($x10^{12}/l$)	Hemoglobin/ *Hemoglobin* (g/l)	Hematocrit / *Hematokrit* (l/l)	MCV/ *MCV* (fl)	MCH/ *MCH* (pg)	MCHC/ *MCHC* (g/l)
Before/ *Pre* ($\bar{x}$ ±SD)	6.31 ± 0.89	147.20 ± 19.61	0.43 ± 0.05	68.53 ± 2.10	23.41 ± 0.61	341.50 ± 6.26
After/ *Posle* ($\bar{x}$ ±SD)	6.60 ± 0.87	150.89 ± 23.57	0.45 ± 0.05	68.09 ± 2.29	22.81 ± 1.23	335.22 ± 22.16

The concentration of serum protein, the serum enzyme values (CK and LDH), as well as creatinine and BUN concentrations were in the extent of normal values for dogs. The serum chemistry analyses of the dogs before and after DST demonstrated significant differences in potassium, sodium and chloride concentrations. Serum potassium concentration significantly decreased in DST (4.81 ± 0.14 mmol/l before DST versus 4.59 ± 0.29 mmol/l after DST; $p = 0.046$). A significant increase in serum sodium concentration was observed after DST in dogs (142.90 ± 3.87 mmol/l before DST versus 143.80 ± 4.13 mmol/l after DST; $p = 0.0187$). As far as serum chloride concentration is concerned, it was significantly increased after DST (107.10 ± 2.33 mmol/l before DST versus 108.50 ± 2.46 mmol/l after DST; $p < 0.001$).

Table 2. Values of serum biochemical constituents in dogs before and after DST
Tabela 2. Vrednosti biohemijskih analiza seruma kod pasa pre i posle DST

Parameter / *Parametar*	Before DST/ *Pre DST*	After DST/ *Posle DST*	Parameter / *Parametar*	Before DST/ *Pre DST*	After DST/ *Posle DST*
Albumin/ *Albumini* (g/l)	24.50±2.72	24.50±3.10	Potassium / *Kalijum* (mmol/l)	4.81±0.13	4.59±0.29*
Protein/ *Proteini* (g/l)	60.70 ±3.30	59.20±4.94	Sodium / *Natrijum* (mmol/l)	142.90±3.87	143.80±4.13*
BUN / *Urea* (mmol/l)	8.78±2.41	8.82±2.53	Calcium / *Kalcijum* (mmol/l)	2.33±0.10	2.31±0.13
Creatinine/ *Kreatinin* (μmol/l)	87.40±12.14	85.80±14.08	Chloride / *Hloridi* (mmol/l)	107.10±2.33	108.50±2.46**
CK/ *CK* (IU/l)	59.90±9.93	60.80±17.72	Bicarbonate/ *Bikarbonati* (mmol/l)	20.20±4.59	20.10±4.01
LDH/ *LDH* (IU/l)	57.20±17.97	55.40±23.98	* (p<0.05)	** (p<0.001)	

A B C



Graph. 1. Serum electrolytes concentrations before and after DST in the dogs (A – serum potassium concentrations; B – serum sodium concentrations; C – serum chloride concentrations)
Graf. 1. Koncentracije elektrolita seruma kod pasa pre i posle DST (A – koncentracije kalijuma; B – koncentracije natrijuma; C – koncentracije hlorida)

DISCUSSION

It is very important to consider shortness of DST protocol in veterinary medicine because of uncooperativity of dogs. Until now, different protocols of DST were reported in canine cardiology. There were protocols of low dose dobutamine infusion of 5 μg/kg/min (Minors and O'Grady, 1998; Koplitz et al., 2004) which lasted 15 minutes, as well as protocols of incremental dobutamine infusion (McEntee et al., 1996, 1998, 2000, 2001; Sousa et al., 2005) which lasted 40 to 75 minutes.

In this study, criteria for choosing DST protocol were appliance in clinical practice. Thus, the minimal dose (7,5 μg/kg/min) was in the therapeutic range for dobutamine dosage; and afterwards it was gradually increased every 3-minute by 5 μg/kg/min. Maximum duration of DST was 24 minutes. Toleration of this DST protocol by the dogs was good.

The CBC changes (RBC, Hgb, PCV, MCV, MCH, MCHC) associated with DST were similar with ones in treadmill running physical exercise (Strasser et al., 1997). However, these CBC changes were not significant.

DST induces significant serum electrolyte changes in dogs. Sodium concentration after DST was significantly higher ($p=0.0187$) than before DST. Increased sodium concentration is in agreement with sodium concentration changes (134.59 ± 9.46 mmol/l vs. 137.12 ± 10.17 mmol/l) in exercise stress testing (Strasser et al., 1997). Chloride concentration was significantly increased ($p < 0.001$) after DST. Opposite, exercise stress testing with treadmill running in study of Strasser et al. (1997) induced decreasing of chloride concentration (122.28 ± 6.19 mmol/l vs. 122.00 ± 6.35 mmol/l). Potassium concentration after DST in dogs was significantly lower ($p=0.046$) than before DST. Increased potassium concentration (4.04 ± 0.27 mmol/l vs. 4.21 ± 0.30 mmol/l) was registered in dogs after treadmill stress testing in study of Strasser et al.(1997). Decreased potassium concentration after DST in dogs is in agreement with DST study in horses (Frye et al., 2003). Dobutamine infusion induces decrease plasma potassium concentration, with a further decrease 10 minutes later. The maximum decrease in potassium occurs in the patients who received the highest dose of dobutamine (Coma-Canella, 1991). This phenomenon has been partially attributed to potassium influx induced by β adrenergic stimulation of the $Na^+ - K^+ - 2Cl^-$ cotransporter. Because of the activity of sodium-potassium–adenosine triphosphatase ion pumps, the opposite occurs with sodium (delCastillo et al., 1999). Chloride levels will follow sodium levels except in the case of acid-base imbalances. On the other hand, activation of adrenergic receptor stimulates adenyl cyclase with resultant increases in cAMP and protein kinase activity. Furthermore, outward potassium current (Ik) is diminished which leads to prolonged repolarization and action potential prolongation (Thomas et al, 2004). As a result, prolong cardiac repolarization may cause early afterdepolarization, thereby initiating development of cardiac arrhythmias.

In this study no arrhythmias were recorded during DST. Although serum electrolytes concentrations have been significantly changed, these changes were not clinically significant. Since this study was performed in healthy dogs, further studies are needed to investigate the possibilities of induction of arrhythmias based on changed electrolytes concentrations, especially potassium, in dogs with cardiac diseases.

CONCLUSION

The conventional DST protocol in dogs leads to serum electrolytes changes. The changes of major electrolytes could be explained by dobutamine effects as β1- adrenergic agonist. Adverse effects associated with hypokalemia in DST need additional study so that safety of DST in dogs could be documented.

REFERENCES

COMA-CANELLA, I.: Changes in plasma potassium during the dobutamine stress test. Int J Cardiol, 33, 55 – 59, 1991.

DELCASTILLO, J.R., AREVALO, J.C., BURGUILLOS, L., SULBARAN-CARRASCO, M.C.: Beta-adrenergic agonists stimulate Na^{+}-K^{+}-Cl^{-} cotransport by inducing intracellular Ca^{2+} liberation in crypt cells. Am J Physiol, 277 (3Pt 1) 563 – 571, 1999.

FRYE, M.A., BRIGHT, J.M., DARGATZ, D.A., FETTMAN, M.J., FRISBIE, D.D., BAKER, D.C., TRAUB-DARGATZ, J.L.: A comparison of dobutamine infusion to exercise as a cardiac stress test in healthy horses. J Vet Intern Med**,** 17 (1) 58 – 64, 2003.

HINDS, J.E., HAWTHORNE, E.W.: Comparative cardiac dynamic effects of dobutamine and isoproterenol in concious instrumented dogs. Am J Cardiol, 36, 894 – 901, 1975.

JEWITT, D., MITCHELL, A., BIRKHEAD, J., DOLLERY, C.: Clinical cardiovascular pharmacology of dobutamine, a selective inotropic cathecholamine. Lancet, 2, 363 – 367, 1974.

KOPLITZ, S.L., MEURS, K.M., BAUMWART, R., NISHIJIMA, Y.: Effect of dobutamine on left ventricular outflow tract dynamics in boxers with soft ejection murmurs. http./www. acvim.org/uploadedFiles/Forum/abstracts/AbstractsComplete.pdf, 2004.

LIANG, C., HOOD, W.B.: Dobutamine infusion in conscious dogs with and without autonomic nervous system inhibition, effects on systemic hemodynamics, regional blood flows and cardiac metabolism. J Pharmacol Exp Ther, 211, 698 – 705, 1979.

LIANG, C., YI, J.M., SHERMAN, L.G., BLACK, J., GAVRAS, H., HOOD, W.B.: Dobutamine infusion in conscious dogs with and without acute myocardial infarction: Effects on systemic hemodynamics, myocardial blood flow, and infarct size. Circ Res, 49, 170 – 180, 1981.

LIMA, R.D.E.S.L., DELORENZO, A., ISSA, A.: Reduced adverse effects with an accelerated dobutamine stress protcol compared wiith the conventional protocol: a prospective, randomiyed, myocardial perfusion scintigraphy study. The International Journal of Cardiovascular Imaging 24, 55 – 59, 2008.

MCENTEE, K., AMORY, H., PYPENDOP, B., BALLIGAND, M., CLERCX, C., MICHAUX, C., JACQMOT, O., ROBERT, F., GERARD, P., POCHET, T., HENROTEAUX, M.: Effects of dobutamine on isovolumic and ejection phase indices of cardiac contractility in conscious healthy dogs. RVS, 64, 45 – 50, 1998.

MCENTEE, K., CLERCX, C., FLANDRE, T., PYNNAERT, C., VAN BENEDEN, R., KETELSLEGERS, J.M., MICHAUX, C., BALLIGAND, M., MISERQUE, N., HENROTEAUX, N.: Hormonal response to dobutamine cardiac stress testing in a conscious canine model of early left ventricular dysfunction induced by chronic rate overload. J of Vet Cardiol, 2, 7 – 14, 2000.

MCENTEE, K., CLERCX, C., PYPENDOP, B., PEETERS, D., BALLIGAND, M.: Car-

diac performance in conscious healty dogs during dobutamine infusion. RVS, 61, 234 – 239, 1996.
MCENTEE, K., CLERCX, C., SOYEUR, D., AMORY, H., MICHAUX, C., FLANDRE, T., JONVILLE, E., PYNNAERT, C., MISERQUE, N., HENROTEAUX, M.: Usefulness of dobutamine stress tests for detection of cardiac abnormalities in dogs with experimentally induced early left ventricular dysfunction. Am J Vet Res, 62, 448 – 455, 2001.
MINORS, S.L., O'GRADY, M.R.: Resting and dobutamine stress echocardiography factors associated with the development of occult dilated cardiomyopathy in healthy Doberman Pincher dogs. J Vet Intern Med, 12, 369 – 380, 1998.
SOUSA, M.G., PEREIRA-NETO, G.B., CARARETO, R., GERARDI, D.G., CAMACHO, A.A.: Assessment of eletrocardiographic parameters in healthy dogs undergoing dobutamine stress testing. Schweiz Archiv Tierheilkunde 147, 541 – 545, 2005.
STRASSER, A., SIMUNEK, M., SEISER, M., HOFECKER, N.G.: Age-dependent changes in cardiovascular nad metabolic responses to exercise in beagle dogs. Zentrablatt fur Veterinarmedizin, 44, 449 – 460, 1997.
THOMAS, G.S., MIYAMOTO, M.I.: Should Simultanious exercise become the standard for adenosine myocardial perfusion imaging. Am J Cardiol, 94 (supl) 3D – 11D, 2004.
VATNER, S.F., MCRITCHIE, R.J., BRAUNWALD, E: Effects of dobutamine on left ventricular performance, coronary dynamics and distribution of cardiac output in concious dogs. J Clin Invest, 53, 1265 – 1273, 1974.
WACKERS, F.J.T.: Which pharmacological stress is optimal. Circulation, 87 (2) 646 – 648, 1993.

PROMENA KONCENTRACIJE ELEKTROLITA SERUMA KOD PASA PODVRGNUTIH DOBUTAMIN STRES TESTU

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Izvod

Cilj ovog rada je da ispita hematološke i biohemijske promene seruma zdravih pasa podvgnutih konvencionalnom protokolu dobutamin stres testa. Ispitivanje je izvedeno na 10 zdravih nemačkih ovčara. Dobutamin stres test je izveden tako što je na svaka 3 minuta doza dobutamina povećavana počevši od 7,5 μg/kg/min do 42,5 μg/kg/min. Uzorci krvi su prikupljani pre i posle dobutamin stres testa, kako bi se uradila analiza kompletne krvne slike i biohemijskih parametara seruma. Statistički značajne promene koncentracije kalijuma ($p<0,05$), natrijuma ($p<0,05$) i hlorida ($p<0,001$) su registrovane nakon dobutamin stres testa. Registrovane promene mogu da se objasne delovanjem dobutamina preko β1 adrenergičnih receptora.

Ključne reči: dobutamin stres test, elektroliti, pas.

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