THE RELATIONSHIP BETWEEN END-TIDAL CO$_2$, MEAN ARTERIAL BLOOD PRESSURE AND NEUROENDOCRINE RESPONSE IN CANINE HAEMORRHAGIC SHOCK

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The relationship between end-tidal CO$_2$ (ETCO$_2$), mean arterial blood pressure (MAP) and neuroendocrine response was investigated in experimentally induced haemorrhagic shock in six anaesthetized, spontaneously breathing dogs. One third of the calculated whole blood volume, i.e. 30 ml/kg was gradually withdrawn in 32 minutes. After 16 minutes, the blood was transfused to the dogs. Blood samples were taken regularly for plasma adrenaline, noradrenaline, beta-endorphin and serum cortisol levels. MAP and ETCO$_2$ decreased simultaneously during the withdrawal period. MAP increased before resuscitation commenced due to the increased sympathetic response, confirmed by high adrenalin levels. ETCO$_2$ remained low, suggesting that ETCO$_2$ reflects changes in cardiac output earlier during resuscitation when compared to MAP. Adrenaline, noradrenaline, beta-endorphin and cortisol levels increased during haemorrhagic shock and slowly decreased during resuscitation. The results of the study proved a good correlation and clinical relevance of ETCO$_2$ and MAP during development of haemorrhagic shock while the difference between ETCO$_2$ and MAP increased during resuscitation, suggesting the influence of sympathetic response, confirmed by increased levels of adrenaline. According to a proven positive correlation between ETCO$_2$ and cardiac output during haemorrhagic shock, the results suggest that ETCO$_2$ may be used as a better indicator of haemodynamic events when compared to MAP during a resuscitation period in haemorrhagic shock.

Key words: dogs, end-tidal CO$_2$, haemorrhagic shock, mean arterial blood pressure, neuroendocrine response

INTRODUCTION

Haemorrhagic shock is the most commonly recognized form of hypovolaemic shock. The loss of blood may be due to trauma, haemorrhagic diathesis, surgery, or organ damage due to disease, or it may be experimental (Hauptman and Chaudry, 1993). The decreased blood volume leads to decreased venous return to the heart, which in turn causes decreased cardiac
output. A variety of homeostatic mechanisms are activated to improve circulating volume and to maximize perfusion of vital organs (Mandell and King, 1998).

Decreased cardiac output activates the vasomotor centre of the medulla oblongata and results in the release of the inhibition of the sympathetic centre and activation of inhibition of the parasympathetic centre. Adrenaline and noradrenaline are released from adrenal medulla, and the direct effects are an increase in heart rate, increased cardiac contractility, and arterial and venous vasoconstriction. The combination of catecholamine stimulation and adrenocorticotropic hormone (ACTH) release increases circulating cortisol that mobilizes substrates necessary for energy production (Day and Bateman, 2006). Beside ACTH, beta-endorphin is released from the pituitary gland during haemorrhagic shock (Tuggle and Horton, 1986). The morphinomimetic properties of beta-endorphin have been implicated in the progressive cardiovascular depression that characterizes the state of shock (Laubie et al., 1977; Moss and Scarpelli, 1981; Lemaire et al., 1978).

Invasive methods of measuring cardiac output have been found in spite of the fact that many aspects were impractical to be carried out in a timely manner and costly for a small animal practice. An alternative to invasive cardiac output monitoring in several conditions is the measurement of ETCO₂. Logarithmic correlation has been established between cardiac output and ETCO₂ during low-flow states such as haemorrhagic shock. At near-normal and increased levels of cardiac output, ETCO₂ no longer reflects blood flow; however when cardiac output levels off at a normal or elevated volume, ETCO₂ can give useful information about the adequacy of ventilation provided that there is little ventilation perfusion mismatch (Ornato et al., 1990).

It is a well known fact that cardiac output and MAP are in disproportion during haemorrhagic shock. Cardiac output follows the decrease in blood volume while MAP is maintained due to increased systemic vascular resistance. In advanced stages of acute haemorrhage these mechanisms are overwhelmed and frank hypotension ensues (Falk et al., 1992).

In the present study, we observed the relationship between ETCO₂, MAP and neuroendocrine response in experimentally induced haemorrhagic shock in spontaneously breathing dogs. As hormonal modulation with cardiovascular response occurs secondary to a decreased cardiac output, we assumed that changes in both plasma adrenaline and noradrenaline concentrations, as well as other stress hormones i.e. beta-endorphin and cortisol, will be in close correlation with MAP. We also assumed that ETCO₂, which is determined primarily by the cardiac output and pulmonary blood flow during haemorrhagic shock, will reflect the changes in blood volume, thus better comparing to mean arterial blood pressure.

MATERIAL AND METHODS

Animals

Six adult, intact male, Beagle dogs, weighing in average 15.2 kg, all free from clinical evidence of disease, were included in the study. Dogs were identified
as DEA 1.1 positive or negative according to canine blood group system, the DEA (Dog Erythrocyte Antigen), using blood group determination assay (The Rapid Vet-H, dmslaboratories inc.). Before the experiment, food was withheld for 12 hours, but unlimited access to water was allowed up to the time of premedication.

**Study design**

Dogs were premedicated with acepromazine (PromAce, Fort Dodge Animal Health) 0.05 mg/kg and butorphanol (Torbugesic, Fort Dodge Animal Health) 0.2 mg/kg, both intravenously. They were left undisturbed 20 minutes, after which they were induced to anaesthesia with thiopentone (Nesdonal, Merial) 6 mg/kg intravenously. The dogs were endotracheally intubated and connected to an anaesthesia machine (Ohmeda VMK) using a circle circuit. Anaesthesia was maintained using halothane (Fluothane, Zeneca Ltd) delivered in oxygen and N₂O in a ratio 1:2 (fractional concentration of inspired O₂ \[FIO₂\] = 0.33) with oxygen flow of 30 ml/kg/min. Dogs were positioned in dorsal recumbency and allowed to breathe spontaneously throughout the procedure. An arterial catheter was introduced percutaneously into the femoral artery for the purpose of monitoring arterial blood pressure and sampling. A catheter was introduced into the left jugular vein for blood withdrawal and sampling of venous blood. After instrumentation (approximately 20 minutes after induction), the baseline measurements were taken (phase 1).

The dogs were bled according to a fixed shock protocol in which one third of calculated whole blood volume, i.e. 30 ml/kg was gradually withdrawn in 32 minutes. The blood was withdrawn in five consecutive phases eight minutes apart (phases 1, 3, 5, 7, 9), immediately after the measurements blood samples were taken. Each blood withdrawal lasted approximately 30 seconds. The blood was kept in a reservoir with anticoagulant solution ACD (acid-citrate-dextrose) at room temperature. 16 minutes after the last withdrawal of blood (phase 13), the first one fifth of shed blood was returned to the dog and the procedure was repeated every eight minutes (phases 15, 17, 19, 21) until all the blood was returned (phase 21; 80 minutes after the baseline measurements). Four minutes later (phase 22), halothane and N₂O were discontinued and the dogs were allowed to breathe 100% oxygen. They were disconnected from the anaesthesia machine and were transported to the recovery room four minutes later (phase 23).

Volume % of halothane (vapour setting) was gradually decreased from 2% at the baseline measurements (phase 1) to 1.5% at 12 minutes after the baseline measurements (phase 4). Halothane was decreased further to 1% at 32 minutes after the baseline measurements (phase 9) and to 0.5% at phase 11. At phase 14, the volume % of halothane was increased back to 1%. The volume % of halothane was gradually increased back to 2% after the fourth volume of shed blood was returned (phase 19; 72 minutes after the baseline measurements).

**Monitoring**

The following parameters were recorded during anaesthesia: heart rate (HR), mean arterial blood pressure (MAP), esophageal body temperature (T) and ECG (Hewlett Packard 78354A), tidal volume (TV) and respiratory rate (RR;
Ohmeda Volume Monitor 5410), end-tidal CO$_2$ (ETCO$_2$) and arterial oxygen saturation measured by pulse oximetry (SpO$_2$; Ohmeda OxiCap 4700). Minute volume of respiration (MV) was calculated from RR and average value of 5 consecutive tidal volumes. Measurements were taken every four minutes throughout the duration of anaesthesia (phases 1 – 23).

**Blood sampling and analysis**

Blood samples were taken from the jugular vein after the measurements at phases 1, 6, 10, 13, 16, 22 and 23. Blood samples for the determination of plasma catecholamines (adrenaline and noradrenaline) and beta-endorphin were collected into cold EDTA tubes. They were kept on ice until centrifuged at 2000 rpm for 10 minutes at 4°C. Immediately after centrifugation the plasma was harvested and stored at -20°C until analysed within two weeks of collection. Adrenaline and noradrenaline concentrations were determined by using commercially available radioimmunoassay kits (Byk Gulden, Germany). Beta-endorphin concentrations were determined by using commercially available radioimmunoassay kits (Nichols Institute Diagnostics, USA).

Samples of venous blood for the determination of serum cortisol concentrations were collected into plain tubes. Once samples were clotted, tubes were centrifuged at 3000 rpm for 10 minutes at 4°C. The serum was separated and stored at -20°C until analysed within two weeks of collection. Serum cortisol was determined by using commercially available radioimmunoassay kits (Immunotech; Beckaman Coulter, United Kingdom).

**Statistical analysis**

Data were analyzed by commercial software (SPSS for Windows). Unless otherwise stated, all reported values are means (± SD). Changes in parameters measured or calculated during the experiment were assessed by ANOVA (Petrie et al., 1999). Pearson correlation coefficients were calculated by the use of bivariate correlations procedure that computes Pearson’s correlation coefficients. A value of p<0.05 was considered significant.

The study protocol was approved by the Ministry of Agriculture, Veterinary Administration of the Republic of Slovenia.

**RESULTS**

Plasma adrenaline concentration increased significantly during haemorrhagic shock and remained so throughout the experiment; however it showed a tendency to decrease after the first one fifth of the shed blood was returned. Plasma noradrenaline concentration showed a similar pattern although it changed later (the highest value was measured not earlier than at phase 16, that is after the second one fifth of shed blood was returned). The increase was significant only at the end of the shock period (phase 13). Plasma beta-endorphin and cortisol levels changed likewise (the highest value was observed at phase 16
after which it decreased); only cortisol plasma concentration was increased significantly (Table 1).

<table>
<thead>
<tr>
<th>Phases of experiment</th>
<th>Adrenaline (nmol/l)</th>
<th>Noradrenaline (nmol/l)</th>
<th>Beta-endorphin (pmol/l)</th>
<th>Cortisol (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.442±0.258</td>
<td>1.570±0.176</td>
<td>3.60±2.57</td>
<td>144.89±66.35</td>
</tr>
<tr>
<td>F6</td>
<td>0.543±0.454</td>
<td>0.905±0.274*</td>
<td>3.90±3.06</td>
<td>289.83±152.88*</td>
</tr>
<tr>
<td>F10</td>
<td>1.108±0.329*</td>
<td>1.544±0.737</td>
<td>4.63±3.45</td>
<td>289.71±139.79</td>
</tr>
<tr>
<td>F13</td>
<td>1.343±0.232*</td>
<td>2.025±0.268*</td>
<td>5.18±3.08</td>
<td>335.24±168.92*</td>
</tr>
<tr>
<td>F16</td>
<td>1.250±0.504*</td>
<td>2.977±1.482</td>
<td>5.29±3.60</td>
<td>385.64±178.48*</td>
</tr>
<tr>
<td>F22</td>
<td>0.760±0.284*</td>
<td>1.840±0.993</td>
<td>4.29±2.88</td>
<td>306.12±176.98*</td>
</tr>
<tr>
<td>F23</td>
<td>0.853±0.260*</td>
<td>2.017±1.504</td>
<td>3.88±2.35</td>
<td>288.54±161.48*</td>
</tr>
</tbody>
</table>

*p significantly different (p < 0.05) from F1

A significant negative correlation \( r = -0.47; p = 0.001 \) was observed between MAP and plasma adrenaline concentration. The correlation was even higher \( r = -0.59; p = 0.0001 \) between ETCO\(_2\) and plasma adrenaline concentration. A negative correlation was observed between plasma noradrenaline concentration and both MAP \( r = -0.36; p = 0.01 \) and ETCO\(_2\) \( r = -0.29; p = 0.032 \), and between plasma beta-endorphin concentration and ETCO\(_2\) \( r = -0.32; p = 0.02 \) (Table 2).

Table 2. Correlation coefficients (r) and probability value (p) between MAP or ETCO\(_2\) and plasma hormone concentrations

<table>
<thead>
<tr>
<th></th>
<th>MAP</th>
<th>ETCO(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td>( r = -0.47 ) ( p = 0.001 )</td>
<td>( r = -0.59 ) ( p = 0.000 )</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>( r = -0.36 ) ( p = 0.010 )</td>
<td>( r = -0.29 ) ( p = 0.032 )</td>
</tr>
<tr>
<td>Beta-endorphin</td>
<td>( r = -0.15 ) ( p = 0.167 )</td>
<td>( r = -0.32 ) ( p = 0.020 )</td>
</tr>
<tr>
<td>Cortisol</td>
<td>( r = 0.079 ) ( p = 0.652 )</td>
<td>( r = -0.231 ) ( p = 0.181 )</td>
</tr>
</tbody>
</table>

ETCO\(_2\) significantly decreased immediately after the withdrawal of the first one fifth of the blood and remained significantly lower throughout the experiment compared to the baseline values, despite it increased each time the shed blood was returned. MAP decreased significantly after the first blood withdrawal and
increased before the next withdrawal indicating a falling and rising pattern with an altogether tendency to decrease. During haemorrhagic shock (phases 10 to 13), MAP slowly increased. The rising and falling pattern was observed when the blood was returned to the dogs (steep rise after the blood transfusion and decline at following phases) with the tendency to increase towards the end of the experiment. MAP was significantly higher when compared to the baseline values at the end of the experiment.

A significant correlation was observed between ETCO2 and MAP ($r = 0.45; p = 0.0001$). The difference between MAP and ETCO2 significantly decreased at phases 4 to 8 when compared to the baseline difference. The difference significantly increased at phase 16 and remained significant until the end of the experiment (Figure 1).

MV of respiration was significantly higher at phases 2 and 7 during the withdrawal period when compared to the baseline values. Another significant increase was observed when the shed blood was returned to the dogs (phases 15
MV did not differ significantly from baseline values during the shock period (Figure 2).

**DISCUSSION**

ETCO₂ monitoring has emerged as a valuable noninvasive monitor of cardiac output under conditions of very low pulmonary blood flow, as occurs during severe haemorrhage. On the other hand, ETCO₂ tends to be determined primarily by the minute ventilation under conditions of normal cardiac output (Ornato *et al.*, 1990). Because minute volume of respiration did not differ significantly from baseline values during the hypovolaemic period in the present study, we may assume that low values of ETCO₂, which decreased significantly as early as after the first blood withdrawal, may be attributed to changes in cardiac output rather than to minute volume of respiration.

MAP and ETCO₂ decreased simultaneously during the blood withdrawal in the present study. MAP began to increase before the commencement of
resuscitation reflecting the activation of sympathetic mechanisms protecting the organism from hypotension during acute haemorrhage. Because of the activation of compensatory mechanisms, including the release of catecholamines from adrenal glands, MAP may be maintained despite severe reductions in cardiac output and systemic perfusion (Falk et al., 1992), which is consistent with high adrenalin levels in the present study.

In the present study the difference between MAP and ETCO₂ decreased before and increased during resuscitation, indicating that ETCO₂ is in a higher correlation with cardiac output (Ornatto et al., 1990) rather than with MAP.

Decreased afferent activity of arterial baroreceptors, atrial volume receptors and chemoreceptors due to decreased effective blood volume in haemorrhagic shock stimulate the sympathetic activity of the vasomotor centre. An excessive excretion of adrenaline from the adrenal glands takes place, while noradrenaline is excreted to a lesser degree (Greco and Stabenfeldt, 1997). In the present study, plasma adrenaline concentration increased significantly during haemorrhagic shock; however it decreased after resuscitation when whole blood was initiated. Plasma noradrenaline concentration changed similarly but later and to a lesser degree compared to plasma adrenaline concentration, the results being consistent with previously published data (Greco and Stabenfeldt, 1997).

Beta-endorphin and adrenocorticotropin (ACTH) are released from the pituitary gland under a variety of stressful conditions including haemorrhagic shock. Chernov et al. (1986) have shown a significant rise in beta-endorphin levels during sublethal haemorrhage in nonhuman primates. The rise in beta-endorphin levels appears to correlate with a fall in cardiac output in primates (Mcintosh et al., 1986) as well as canines (Daly et al., 1987) haemorrhagic shock model. In the present study, beta-endorphin levels increased during haemorrhagic shock although the increase was not significant. However, a negative correlation \( r = -0.32; p = 0.02 \) was observed between beta-endorphin levels and ETCO₂ while beta-endorphin levels and MAP did not correlate. Because cardiac output was not measured in the present study, we may assume that according to the already known correlation between beta-endorphin and cardiac output (Mcintosh et al., 1986; Daly et al., 1987) and between cardiac output and ETCO₂ during haemorrhagic shock (Ornato et al., 1990), ETCO₂ in the present study detected early changes in cardiac output.

ACTH secretion by the anterior pituitary gland is followed within minutes by greatly increased adenocortical secretion of cortisol (Guyton, 1991). Cortisol levels were relatively high at the beginning of the experiment comparing to the values observed in non-stressed dogs (Kolevska et al., 2003). These values were most probably related to anaesthesia, which is stressful by itself. According to previously published data (Guyton, 1991), serum cortisol concentration significantly increased during haemorrhagic shock in the present study and remained increased until the end of the experiment, although it showed a tendency to decrease after resuscitation when whole blood was initiated. No correlation was observed between cortisol and ETCO₂ or MAP.

In conclusion, this study has demonstrated a significant correlation between ETCO₂ and MAP during blood loss, which may be clinically associated to early
stages of haemorrhagic shock. The difference between ETCO₂ and MAP increased before and remained high during the resuscitation period. Although spontaneous ventilation was used in the present study, the results suggest that minute volume of respiration did not influence ETCO₂ values significantly, therefore the authors assume that ETCO₂ reflected the changes in cardiac output. Monitoring of resuscitation success of haemorrhagic shock with MAP only can be misleading, thus ETCO₂ measurements are highly recommended during the management of haemorrhagic shock regardless whether spontaneous breathing or controlled ventilation is used.

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ODNOS IZMEĐU PARCIJALNOG PRITISKA UGLJEN DIOKSIDA (ETCO₂), SREDNJEG ARTERIJSKOG PRITISKA (SAP) I NEUROENDOKRINOG ODGOVORA U TOKU HEMORAGIČNOG ŠOKA PASA

SADRŽAJ

U ovom radu su uzneti rezultati proučavanja odnosa između parcijalnog pritiska ugljen dioksida na kraju izdisaja (ETCO₂), srednjeg arterijskog pritiska (SAP) i neuroendokrinog odgovora tokom eksperimentalno izazvanog hemoragičnog šoka u šest anesteziranih pasa sa spontanim disanjem. Psima je u toku 32 minuta, postepeno uzimano 30 ml/kg krvi, što iznosi 1/3 celokupne izračunate zapremine. Nakon 16 minuta, započeto je vraćanje krvi psima. U redovnim vremenskim razmacima uzimani su uzorci krvi za određivanje koncentracije adrenalina, noradrenalina i beta endorfina u plazmi i kortizola u serumu. U periodu uzimanja krvi, SAP i ETCO₂ su se istovremeno smanjivali. SAP se povećao pre početka vraćanja krvi, najverovatnije zbog povećanog simpatikusnog odgovora, koji je utvrđen na osnovu visokih vrednosti koncentracije adrenalina. ETCO₂ je ostao smanjen, što ukazuje da ETCO₂ odražava promene minutnog volumena srca nešto ranije u toku lečenja hemoragičnog šoka u poređenju sa SAP-om. Koncentracije adrenalina, noradrenalina, beta endorfina i kortizola su se povećavale za vreme hemoragičnog šoka i polako se smanjivale tokom postupka vraćanja krvi.

Rezultati studije su dokazali dobru korelaciju i klinički značaj ETCO₂ i SAP-a u razvoju hemoragičnog šoka, međutim razlika između ETCO₂ i SAP-a se povećava tokom vraćanja krvi ukazujući na uticaj simpatikusnog odgovora, što je potvrdjeno povišenom koncentracijom adrenalina. Pozitivna korelacija između ETCO₂ i minutnog volumena srca za vreme hemoragičnog šoka ukazuje da bi ETCO₂ mogao da posluži kao bolji indikator hemodinamičkih događaja u poređenju sa SAP-om tokom perioda vraćanja krvi (transfuzije) pri terapiji hemoragičnog šoka.