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Persistent reduced oxygen requirement following blood transfusion during recovery from hemorrhagic shock

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ABSTRACT

Our study intended to determine the effects on oxygen uptake ($\dot{V}_{O_2}$) of restoring a normal rate of O_2 delivery following blood transfusion (BT) *after* a severe hemorrhage (H). Spontaneously breathing urethane anesthetized rats were bled by removing 20 ml/kg of blood over 30 min. Rats were then infused with their own shed blood 15 min after the end of H. At mid-perfusion, half of the rats received a unique infusion of the decoupling agent 2,4-dinitrophenol (DNP, 6 mg/kg). $\dot{V}_{O_2}$ and arterial blood pressure (ABP) were continuously measured throughout the study, along with serial determination of blood lactate concentration [La]. Animals were euthanized 45 min after the end of reperfusion; liver and lungs were further analyzed for early expression of oxidative stress gene using RT-PCR.

Our bleeding protocol induced a significant decrease in ABP and increase in [La], while $\dot{V}_{O_2}$ dropped by half. The O_2 deficit progressively accumulated during the period of bleeding reached -114 ± 53 ml/kg, just before blood transfusion. Despite the transfusion of blood, a significant O_2 deficit persisted (-82 ± 59 ml/kg) 45 min after reperfusion. This slow recovery of $\dot{V}_{O_2}$ was sped up by DNP injection, leading to a fast recovery of O_2 deficit after reperfusion, becoming positive ($+460 \pm 132$ ml/kg) by the end of the protocol, supporting the view that O_2 supply is not the main controller of $\dot{V}_{O_2}$ dynamics *after* BT. Of note is that DNP also enhanced oxidative stress gene expression (up-regulation of NADPH oxidase 4 in the lung for instance). The mechanism of slow recovery of O_2 requirement/demand following BT and the resulting effects on tissues exposed to relatively high O_2 partial pressure are discussed.

1. Introduction

During a severe hemorrhagic shock (H), O_2 consumption ($\dot{V}_{O_2}$) decreases (Dunham et al., 1991; Rixen and Siegel, 2005; Siegel et al., 2003). The theory put forward to account for this reduction in O_2 consumption is that the decline in O_2 delivery rate (DO_2) below a 'critical' threshold (Vincent and De Backer, 2004) prevents the normal activity of the mitochondrial electron chain, leading to a deficit in ATP production—i.e. not enough O_2 is delivered to sustain mitochondrial ATP production. As a result, an oxygen deficit develops during H, which is assumed to be "repaid" following the restoration of O_2 delivery rate after blood transfusion. This view is supported by studies suggesting that the payment of O_2 debt, i.e. the total deficit in oxygen during and following the period of hemorrhage, is affected by the volume, quality and administration rate of fluids infused during the reperfusion phase (Siegel et al., 1997, 2003). Likewise, in a dog model of hemorrhagic shock and reperfusion, resuscitation with a hemoglobin-based oxygen carrier allowed a faster recovery of base deficit and lactates compared to colloids, despite comparable infused volumes (Driessen et al., 2003). One may infer from these studies that during and following hemorrhage, O_2 availability is the main controller of oxygen consumption in the tissues.

However a significant, albeit variable, part of the reduction in $\dot{V}_{O_2}$ *during* H, can also be accounted for by a reduction in O_2 *demand*. This is made possible via a decrease in cardiac output, blood flow redistribution (Vatner, 1974), reduction in non-shivering thermogenesis (Gautier, 1996b; Mortola and Matsuoka, 1993), shift to non-essential cellular metabolism and cessation of non-vital enzymatic activities (Hochachka et al., 1996). We previously found that *during* a severe hemorrhage, which induces a significant O_2 deficit and blood lactate accumulation, the injection of DNP increases $\dot{V}_{O_2}$, ventilation and cardiac output (Haouzi and Van de Louw, 2013). This observation led to the contention that 1—O_2 deficit is produced during hemorrhage well before O_2 delivery is limited, 2—the reduction in O_2 demand produced by the hemorrhage contributes to the

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reduction in O_2 transport (ventilation and cardiac output), akin to the response to hypoxia (Gautier, 1996b; Mortola and Matsuoka, 1993), creating a vicious circle in terms of peripheral gas exchange (Haouzi and Van de Louw, 2013).

This reduction in O_2 demand may well persist after the restoration of O_2 supply as the mechanisms of O_2 sparing may have a different time constant than that of O_2 delivery rate. O_2 debt can be repaid much slower than expected from DO_2 kinetics due to the development of a genuine autonomous mitochondrial dysfunction (Griffiths and Halestrap, 1995). If true, the kinetics of $\dot{V}_{O_2}$ recovery during reperfusion will not only be dictated by the restoration of adequate O_2 delivery rate, but will also be controlled by the ability of mitochondria to recover their normal function, regardless of how much O_2 is present. Whether or not the "repayment" of O_2 debt following the treatment of H is governed by the progressive recovery of normal cellular energetics (and O_2 demand) or is primarily dictated by the amount of oxygen available remains unclear. This question is essential, as the production of free radicals has been linked to the activity of the electron chain in keeping with the level of O_2 availability, i.e. a low activity (associated to high electrochemical mitochondrial gradient of proton) may result in an increase in free radicals production (Brand and Esteves, 2005; Criscuolo and Bouillaud, 2009; Hausenloy et al., 2004; Minners et al., 2000). Free radicals accumulation could therefore be markedly amplified in condition of low O_2 demand and high DO_2.

The objective of this study was to determine whether mitochondrial O_2 availability is a limiting factor for O_2 debt repayment during the reperfusion phase of a hemorrhagic shock in rats. To test this hypothesis, we studied the kinetics of $\dot{V}_{O_2}$ along with the temporal profile and magnitude of O_2 deficit and O_2 debt repayment during reperfusion following hemorrhagic shock in a rat model. The effects of 2,4-dinitrophenol (DNP), a mitochondrial uncoupling agent, intended to increase the proton transfer toward the inner mitochondrial membrane, and hence oxygen demand, were investigated during blood transfusion. We assessed whether 1—DNP, administered during reperfusion, speeds up the repayment of O_2 debt, 2—the resulting mitochondrial "decoupling" could lead to a reduction in lactate production and oxidative stress through production of free radicals in the liver or the lungs and early change in activity of genes involved in the response.

2. Methods

2.1. Animal preparation

After approval by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee, sixteen adult Sprague-Dawley rats (470 ± 60 g) were studied: anesthesia was induced with 3.5% isoflurane in O_2 followed by intra-peritoneal injection of 1.2 g/kg of urethane (Sigma-Aldrich) as previously described (Haouzi and Van de Louw, 2013). The animals were tracheotomized and the tracheostomy was connected to a small dead space two-way valve. The inspiratory port of the valve was connected to a calibrated pneumotachograph (Hans Rudolph, KS, USA, 8420 series) to measure inspiratory flow. A polyethylene PE-50 catheter was inserted into the left femoral artery for blood withdrawal and arterial blood pressure (ABP) monitoring (Cybersense, Nicholasville, KY, USA). A similar catheter was placed in the right jugular vein. Arterial blood gases (ABG) partial pressures, along with the concentrations of lactic acid, were determined using an i-STAT 1 blood gas analyzer (Abaxis, Union City, CA, USA).

2.2. Measurements and data analysis

The rats were breathing spontaneously room air during the entire protocol. Their body temperature was maintained with a heating pad to 36–37 °C in baseline conditions. The temperature of the pad was however kept unchanged throughout the bleeding and reperfusion both in control conditions and following DNP. The inspiratory flow ($\dot{V}$) and arterial pressure signals were digitized

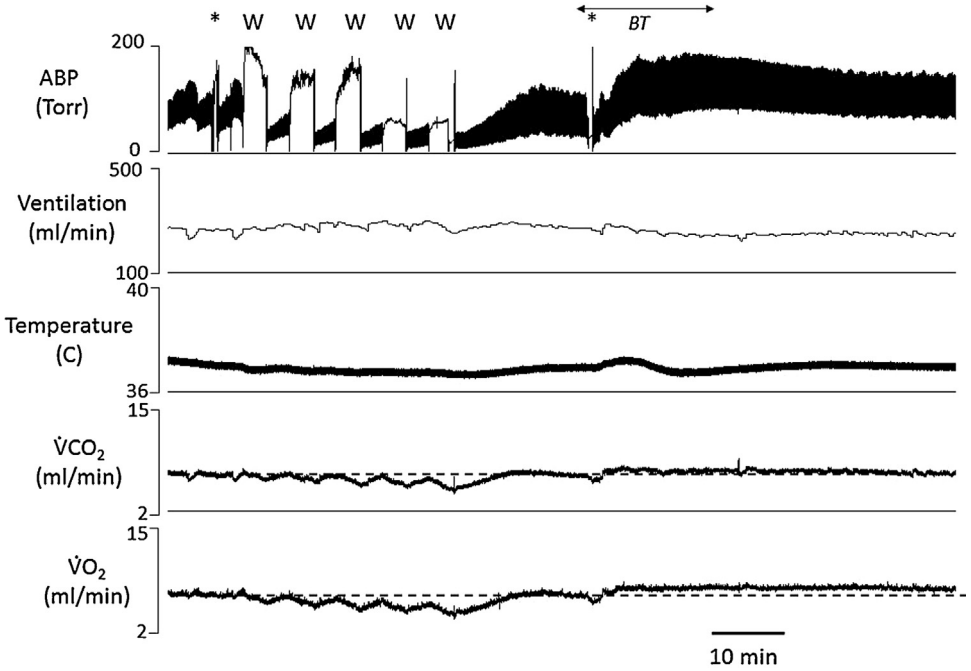

Fig. 1. Example of a recording obtained during acute hemorrhage and following blood transfusion (BT) in one control rat. Arterial blood pressure (ABP), minute ventilation, body temperature (*T*), carbon dioxide production ($\dot{V}_{CO_2}$) and oxygen uptake ($\dot{V}_{O_2}$) are displayed. Interruptions in ABP recording are due to blood withdrawal during each of the bleeding periods (*W*) or blood gas sampling (*). Bleeding induced a drop in arterial pressure, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ and a small decrease in minute ventilation. Following transfusion (BT), all parameters returned to normal with a slow recovery of O_2 debt. Note that body temperature, which slightly decreased during the bleeding period retuned progressively to normal following blood infusion.