Venoarterial CO\textsubscript{2} difference during regional ischemic or hypoxic hypoxia

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Vallet, Benoît, Jean-Louis Teboul, Stephen Cain, and Scott Curtis. Venoarterial CO\textsubscript{2} difference during regional ischemic or hypoxic hypoxia. \textit{J Appl Physiol} 89: 1317–1321, 2000.—To test the role of blood flow in tissue hypoxia-related increased veno-arterial PC\textsubscript{O}{\textsubscript{2}} difference (\(\Delta\text{PCO}_2\)), we decreased O\textsubscript{2} delivery (DO\textsubscript{2}) by either decreasing flow [ischemic hypoxia (IH)] or arterial P\textsubscript{O}{\textsubscript{2}} [hypoxic hypoxia (HH)] in an in situ, vascularly isolated, innervated dog hindlimb perfused with a pump-membrane oxygenator system. Twelve anesthetized and ventilated dogs were studied, with systemic hemodynamics maintained within normal range. In the IH group (\(n = 6\)), hindlimb DO\textsubscript{2} was progressively lowered every 15 min by decreasing pump-controlled flow from 60 to 10 ml\textperkg\textsuperscript{1}min\textsuperscript{-1}, with arterial P\textsubscript{O}{\textsubscript{2}} constant at 100 Torr. In the HH group (\(n = 6\)), hindlimb DO\textsubscript{2} was progressively lowered every 15 min by decreasing P\textsubscript{O}{\textsubscript{2}} from 100 to 15 Torr, when flow was constant at 60 ml\textperkg\textsuperscript{1}min\textsuperscript{-1}. Limb DO\textsubscript{2}, O\textsubscript{2} uptake (VO\textsubscript{2}), and \(\Delta\text{PCO}_2\) were obtained every 15 min. Below the critical DO\textsubscript{2} (DO\textsubscript{2}\textsubscript{crit}), reducing blood flow acts as a confounding variable and results in difficulties in drawing any definitive conclusion on the meaning of increased \(\Delta\text{PCO}_2\) in hypoxia. Moreover, to date, this issue has been addressed exclusively for the whole body and not at the organ level. The aim of this study was to evaluate the \(\Delta\text{PCO}_2\) in a regional model of progressive tissue hypoxia produced by decreasing either flow or Ca\textsubscript{CO}{\textsubscript{2}}.

METHODS

Animal preparation. This study was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Dogs of either sex and mixed breed were used. All animals were initially anesthetized with intravenous pentobarbital sodium (30 mg/kg) and intubated with a cuffed endotracheal tube. Catheters were inserted into the pulmonary artery (via the internal jugular vein) and common carotid artery for continuous measurement of vascular pressures and blood sampling. Lamps suspended above the operating table were used to maintain core temperature near 37°C. Standard limb leads were used to obtain heart rate continuously by means of a cardiotachometer (type 9857 cardiotachometer coupler, Beckman Instruments, Schiller Park, IL).

Arterial inflow (Q) and venous outflow from the left hindlimb were isolated, as previously described (2). In brief, the
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probal 10 cm of the femoral nerve, artery, and vein were dissected free in the groin, and all vascular branches were tied off. Venous outflow from the limb was restricted to the femoral vein by tourniquet technique. With the use of a spinal needle as an introducer, a nylon cord was passed through the limb on each side of the femur, high in the groin. The ends of the two cords were crossed outside of the leg, both posteriorly and anteriorly, and tied tightly, with the femur acting as an anchor. The isolated femoral vessels and nerve were excluded from this tourniquet. Circulation to the paw was excluded by another tourniquet at the ankle. With these measures, ~95% of the effluent blood flow in this preparation can be attributed to muscle (2). To prevent collateral arterial flow to the hindlimb, the left deep circumflex and internal and external iliac arteries were ligated through a midline abdominal incision. Before ligation of these vessels, the femoral artery of the left leg was perfused from the controlateral femoral artery. Arterial isolation and reactive hyperpermia were documented to be present in all animals at the beginning of each experiment by excluding the femoral artery for 30 min. KvCl was given intravenously at a dose of 1,000 U/kg before cross perfusion was initiated. Blood flow from the left femoral vein was returned to a reservoir positioned above, and connected to, the right femoral vein. After each experiment, the left femoral artery was injected with India ink, and the muscle that stained black was dissected free and weighed. Leg blood flow, Do2, and Vo2 were reported per kilogram of muscle mass.

A roller occlusive pump directed blood flow from the right hindlimb femoral artery to the femoral artery of the vascularly isolated left hindlimb. A sampling port and pressure transducer were placed in this circuit proximal to the limb. A membrane oxygenator (model 0800–2A, Sci Med) was infused in the perfusion circuit. A gas flow mixer (model GP-3, Cameron Instruments) supplied O2, N2, and CO2 to the oxygenator, as needed, to produce normoxia or hypoxia with normocapnia in the blood supply to the hindlimb. A water bath warmed the oxygenator so that perfusion to the isolated hindlimb was at 37°C after heat loss through the tubing. After the hindlimb preparation was complete, 20 mg of cyanoc chloride was given intramuscularly and a continuous infusion of 0.1 mg·ml⁻¹·min⁻¹ was begun. Mechanical ventilation was started at 10 breaths/min with a tidal volume of 100 ml. The inspired fraction of O2 was 0.35, and the solubility coefficient, 0.0031 ml O2·dl⁻¹·Torr PO2⁻¹. Cardiac output was calculated by dividing whole body Vo2 by the difference in CaO2 and CvO2. All values were reported per unit of body weight.

Experimental protocol. After all pressures and flows were stable for at least 30 min, the experiment began with a 30-min control period, during which measurements were obtained every 15 min. In the progressive ischemic hypoxia (IH) group, Q was then decreased every 15 min to produce Q values of ~60, 45, 30, 20, 15, and 10 ml·kg⁻¹·min⁻¹. In the hypoxic hypoxia (HH) group, Q was set at 60 mg·kg⁻¹·min⁻¹ and limb Do2 was reduced by decreasing arterial PO2 from 100 to ~15 Torr (i.e., CaO2 of 17 to 2 ml O2/100 ml in eight steps at 15-min intervals. A flow rate of 60 ml·kg⁻¹·min⁻¹ was chosen for progressive hypoxia because it is within the range of resting blood flow to normal skeletal muscle and for the practical reason that a moderate flow was necessary to achieve the desired low PO2 values using the membrane oxygenator. Po2, PvO2, CvO2, CaO2, CvCO2, arterial pH (pHv), and venous pH (pHV) were determined every 15 min, 15 min after the change in hindlimb arterial blood flow or PO2.

ΔPCO2 was calculated as PVCO2 – PaCO2 and ΔpH as pHv – pH. Hindlimb CO2 production (VCO2) was calculated as the product of Q and the difference between CvCO2 and CaCO2. Difference in CO2 content was calculated with the McHardy equation [as proposed by Neviere et al. (6): CvCO2 – CaCO2 = 11.02[(PVCO2–0.3966) – (PaCO2–0.3966)] – (15 – Hb)0.015 (PVCO2 – PaCO2) – (95 – SaO2) 0.064. Hindlimb Vo2 was calculated as the product of Q and the arteriovenous difference in O2 content. Hindlimb respiratory exchange ratio (R) was the ratio of VCO2 to VO2.

Hindlimb Do2 was calculated as the product of Q and CaO2. O2 extraction ratio (ERo2) was calculated as the ratio of Vo2 to Do2. For each experiment, regression lines were fitted to the delivery-independent and -dependent portions of the delivery-uptake curve using a dual-line, least squares method (7). The intercept of these two lines defined the critical Do2 (Do2 crit), that is, the delivery at which Vo2 began to fall with any further decline in Do2.

Statistics. Data were analyzed within and between groups using repeated-measures ANOVA and Newman-Keuls test. Paired and unpaired t-tests were used, as appropriate, for one-time comparisons. Statistical significance was accepted at P < 0.05 for all comparisons.

RESULTS

We wished to maintain systemic hemodynamics within normal range so we could examine the direct local effects of ischemia and hypoxia on the hindlimb without confounding baroreceptor or chemoreceptor influence. Systemic hemodynamics and O2 parameters remained stable throughout the study without any between-group differences. Cardiac output averaged 136 ± 6 (SE) ml·kg⁻¹·min⁻¹ for the 12 dogs. PaO2 was 82 ± 2 Torr, and PaCO2 was 34 ± 2 Torr. Mean arterial pressure was 128 ± 2 Torr and systemic Vo2 was 6.67 ± 0.07 ml·kg⁻¹·min⁻¹. Hematocrit was 39.0 ± 0.4%. These values are typical for paralyzed, pentobarbital sodium-anesthetized dogs.

Figures 1 and 2 depict the changes seen in hindlimb Vo2 and ERo2 as Do2 was decreased by progressive IH or HH. In both groups, the Vo2-to-Do2 graph describes the typical biphasic relationship. Mean Do2 crit was
slightly higher in HH than in IH, but the difference was not statistically significant. ER O2 at D˙O2 crit was significantly larger in IH than in HH (79 ± 6 vs. 66 ± 4%, respectively). Venous PO2 at D˙O2 crit (Fig. 3) was not different between groups (23 ± 1 and 21 ± 2 Torr in IH and HH, respectively). For the lowest D˙O2 obtained, PVO2 was significantly higher in IH than in HH (15 ± 1 and 9 ± 2 Torr, respectively). Beyond D˙O2 crit, ER O2 rose continuously and quite similarly in both groups, reaching a maximal extraction ratio of ~85–90%.

Figure 4 depicts the changes seen in hindlimb V˙CO2 as D˙O2 was decreased by progressive IH or HH. In both groups, the VCO2-to-D˙O2 graph describes a very similar biphasic relationship. The hindlimb respiratory exchange ratio (R) increased in both groups, with a trend to decrease by the end of the experiment in HH (Fig. 5).

ΔPCO2 significantly increased in IH but did not change in HH (Fig. 6). The increase in ΔPCO2 in IH occurred before reaching D˙O2 crit. At D˙O2 crit, ΔPCO2 approached 16 Torr. There was no evidence of changes in the slope of the ΔPCO2-to-D˙O2 relationship. ΔpH increased significantly only in IH (Fig. 7).

DISCUSSION

The main result of this study is that occurrence of an increased ΔPCO2 during ischemia is related to decreased blood flow and impaired CO2 washout. Dysoxia per se is not sufficient to increase ΔPCO2. In presence of a constant flow, dysoxia with CO2 generated from anaerobiosis does not promote ΔPCO2 widening.

Tissue dysoxia occurs when D˙O2 is inadequate to support O2 demand (4, 8). O2 represents the terminal electron acceptor for oxidative phosphorylation. In the absence of adequate D˙O2, the intermediates in the electron transport system are converted to their reduced states, and electron transport is compromised (4). In response to declines in cellular D˙O2, the tissues employ a series of responses to maintain a balance between ATP production (main cellular energy source) and cellular energy needs. The predominant mechanism is an increase in ER O2 of capillary blood (ER O2 = VO2/D˙O2). However, with severe decreases in D˙O2, com-
pensory increases in ER O₂ may not be sufficient to provide the mitochondria with the O₂ required to sustain aerobic metabolism. The cells must then use anaerobic sources of energy to produce ATP, resulting in the generation of lactate and H⁺ ions. In our study, we did not measure lactate production. However, information gained from the V˙O₂-to-D˙O₂ relationship clearly identifies the onset of tissue dysoxia in our hindlimb preparation. Maximal O₂ extraction was comparable in both groups, meaning that physiological responses to impaired O₂ delivery were similarly present in IH and HH. Also, V̇O₂ fell to the same level in both groups by the end of the experiment (∼1 ml O₂·kg⁻¹·min⁻¹), suggesting that a similar severity of dysoxia was reached. We can assume then that HH and IH were comparable in terms of dysoxia. Moreover, dysoxia started at very similar D˙O₂ crit in IH and HH, excluding any possibility of an earlier O₂ debt accumulation in one group that was responsible for a larger CO₂ accumulation.

Oxidative phosphorylation results in the formation of CO₂ and water. When D˙O₂ is progressively decreased below D˙O₂ crit, this is followed by 1) a decrease in tissue oxidized CO₂ and aerobic CO₂ production and 2) an increase in H⁺ concentration associated with tissue CO₂ production resulting from cellular buffering by bicarbonates. Total CO₂ production (VCO₂) beyond D˙O₂ crit is, therefore, the sum of decreased aerobic CO₂ production and increased anaerobic CO₂ production. VCO₂ is related to V̇O₂, i.e., VCO₂ = R × V̇O₂, with R being stable and principally affected by the fuel source used for aerobic metabolism (3, 10). Anaerobic sources of CO₂ may, however, increase R when D˙O₂ is lowered beyond D˙O₂ crit. This was observed by Cohen et al. (3) in hemorrhaged pigs; airway CO₂ production decreased during hemorrhage but less than V̇O₂, and, consequently, R increased. Our results are consistent at the organ level; when flow and D˙O₂ were progressively decreased (IH), V̇CO₂ decreased, but R increased, suggesting some production of anaerobic CO₂. When flow was kept constant while CaO₂ was decreased (HH), we observed a similar decrease in VCO₂, with a trend for an increase in R. Whatever the increase in R, we must admit, however, that anaerobic sources of CO₂ are much less important than aerobic ones because V̇CO₂ consistently and dramatically decreased when D˙O₂ was lowered beyond D˙O₂ crit. This occurred similarly in IH and HH, suggesting an absence of gross difference in VCO₂ for these two forms of hypoxia.

Besides aerobic and anaerobic production of CO₂, two other factors affecting ΔPco₂ are CO₂ dissociation curve and tissue blood flow. The CO₂ dissociation curve is influenced by the saturation of hemoglobin with O₂, a phenomenon known as the Haldane effect (12). The lower the saturation of hemoglobin with O₂, the larger the CO₂ saturation of hemoglobin for a given Pco₂. This might account for a smaller ΔPco₂ in HH, a situation in which larger hemoglobin deoxygenation would increase the blood's ability to carry CO₂. The similar value of PvO₂ at ḊO₂ crit when ΔPco₂ is already larger in IH than in HH, limits this explanation above ḊO₂ crit. Below ḊO₂ crit, the Haldane effect may contribute, however, in magnifying the difference in ΔPco₂ that was observed between HH and IH. This would explain why...
R tends to rapidly decrease by the end of the experiment in HH. VCO₂ decreases more rapidly than VO₂ because more CO₂ is transported by red blood cells.

For a given tissue CO₂ production, a lower blood flow must be associated with a higher PVCO₂. In this study, there was a clear inverse linear relation between hindlimb PVCO₂ and blood flow. Because ∆PCO₂ did not increase in HH, despite comparable levels of tissue dysoxia, decreased blood flow appears to be another cause of the ∆PCO₂ widening observed in the IH group. Increased PVCO₂ was associated with a decrease in pHv and a widening in ΔpH in the IH group. pHv remained almost constant in HH. These results suggest that PVCO₂ was the primary determinant of pH in this model and that respiratory acidosis very likely accounts for expanding ΔpH.

During hindlimb ischemia in this study, ΔPCO₂ was ~16 Torr at the onset of tissue dysoxia. This value is similar to values found in experimental models of progressive hemorrhage or tamponnade (1, 13), in which whole body ΔPCO₂ varied from 12.9 (13) to 14.9 Torr (11) at Do₂ crit. However, in contrast to previous studies done in the whole animal (1, 11, 13), this value cannot be easily determined in our experiments by considering a brisk increase on the ΔPCO₂-to-Do₂ relationship and cannot provide a useful tool to determine Do₂ crit. If ΔPCO₂ is ~15 Torr or larger at the systemic or regional level, one may say that there is a great risk of dysoxia associated with a decrease in flow; if ΔPCO₂ is <15 Torr, one may say nothing about the presence or absence of dysoxia. If we assume that a PCO₂ gradient of 5 Torr exists between the tissues and the venous blood, a ΔPCO₂ of 15 Torr is compatible with the 20 Torr tissue-to-artery ΔPCO₂ value that represents a situation at risk of dysoxia, as determined in a mathematical model by Schlichtig and Bowles (9).

In summary, in the isolated hindlimb model, lowering Do₂ by decreasing flow results in an increased ΔPCO₂, whereas lowering Do₂ by decreasing blood oxygenation does not affect ΔPCO₂. For the first time, we demonstrated that absence of increased ΔPCO₂ does not preclude the presence of tissue dysoxia.

REFERENCES


