Intravenous pyruvate prolongs survival during hemorrhagic shock in swine

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Mongan, Paul D., John L. Fontana, Rouyan Chen, and Rolf Bünger. Intravenous pyruvate prolongs survival during hemorrhagic shock in swine. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2253–H2263, 1999.—Pyruvate improves cellular and organ function during hypoxia and ischemia and stabilizes the NADH redox state and cytosolic ATP phosphorylation potential. In this in vivo study, we evaluated the effects of intravenous pyruvate on cardiovascular and neocortical function, indexes of the cytosolic redox state (lactate/pyruvate ratio, L/P) and cellular energy state (adenosine and degradative products hypoxanthine and inosine, ADO + HX + Ino) during controlled arterial hemorrhage (40 mmHg) in sedated swine (45 kg). NaCl pyruvate was infused 1 h before (1 g·kg⁻¹·h⁻¹) and 2 h during (0.5 g·kg⁻¹·h⁻¹) hemorrhage to attain arterial pyruvate levels of 6 mM. Volume (0.9% NaCl) and osmotic (10% NaCl) effects were matched in controls. Time to peak hemorrhage (57 min) and peak hemorrhage volume (43 ml/kg) were similar in all groups. The volume and osmotic groups experienced spontaneous cardiovascular decompensation between 60 and 90 min, with an average time until death of 82.7 ± 5.5 and 74.8 ± 8.2 min. In contrast, survival in the pyruvate group was 151.2 ± 10.0 min (P < 0.001). During hemorrhage, the pyruvate group had better cardiovascular and cerebrovascular function with significantly higher systemic and cerebral oxygen consumption and less attenuation of the amplitude and frequency of the electrocorticogram. In addition, pyruvate prevented metabolic acidosis and stabilized the L/P. Pyruvate slowed the rise in neocortical microdialysis levels of ADO + HX + Ino, and prevented the net efflux of ADO + HX + Ino into the sagittal sinus. The findings reveal considerable metabolic and functional enhancement by pyruvate during severe hemorrhagic shock with a 75-min delay in spontaneous cardiovascular decompensation and death.

ischemia tolerance; microdialysis; cerebral blood flow; cardiovascular decompensation

AFTER THE MAXIMUM hemorrhage volume is reached during severe experimentally controlled arterial hemorrhage, if the shed blood or other volume is not infused, there is a rapid decrease in the mean arterial pressure (MAP), resulting in life-threatening hypotension and cardiovascular collapse. This decompensatory phase is characterized by a decrease in the peripheral vascular resistance, insensitivity to α-adrenergic agonists, and irreversible loss of vascular contractility (32, 36). Although not fully elucidated, the mechanisms for these vascular changes are primarily related to K⁺-channel activation (27, 33). This can be caused by increases in nitric oxide (32) or decreases in cytosolic ATP, especially in combination with acidosis (25, 27). Whereas experimental strategies to prevent or delay vascular decompensation have focused on the inhibition of nitric oxide synthase or K⁺-channel activation (27, 36), there has been little focus on minimizing cellular energetic deterioration during hemorrhagic shock. In vitro studies have shown that pyruvate, a metabolic substrate, provides remarkable protection of organ and cellular energetics and function during hypoxia, ischemia, or reperfusion (7, 11, 18, 21). Because hypotension, systemic acidosis, and cellular de-energization cause cardiovascular failure during hemorrhagic shock, we investigated the hypothesis that pyruvate administered during hemorrhagic shock could enhance organ tolerance to ischemia and delay cardiovascular collapse.

Pyruvate in millimolar concentrations can improve cellular function during metabolic stress by decreasing the cytoplasmic redox potential ([NADHc] /[NADc ¹]) and maintaining the cellular phosphorylation potential ([ATP]/[ADP][P₅]). During hemorrhagic shock, critical decreases in oxygen delivery cause an increase in cytosolic redox ratio (lactate/pyruvate [NADHc]/[NADc ¹]), depletion of the ATP pool, a fall in the cytosolic phosphorylation potential, and cellular de-energization (36). Substrate-induced changes in the redox state have beneficial effects on both the contractile state and energetics of myocardial and vascular smooth muscle (VSM) (3, 22, 28). A pyruvate-induced decrease in [NADHc]/[NADc ¹] favors an increase in the phosphorylation potential due to coupling of the cytoplasmic redox state to the phosphorylation potential ([ATP]/[ADP][P₅]) through the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase system (5, 28). A second positive effect of pyruvate on oxidative metabolism is improvement in mitochondrial energetics by increasing the driving force for ATP production, mitochondrial NADH (NAD₇₅₄). More important than the overall ATP content, however, is the phosphorylation potential. It is the thermodynamically relevant metabolite ratio of [ATP]/[ADP][P₅] that readily responds to physiological, metabolic, and pathological conditions (7, 13, 16, 28). For example, vital enzyme functions and ionic pump capabilities are stoichiometrically linked to the cytosolic phosphorylation potential (13, 22). Thus maintaining [ATP]/[ADP][P₅] within physiological limits is considered essential for cellular adaptation, integrity, and function, as this ratio has been shown to directly correlate with recovery from ischemic stress (4, 13). Indeed, the provision of exogenous pyruvate to both the heart and VSM has been shown to cause anaplerosis of the citric acid cycle, increase NADH₇₅₄, oxygen consumption, and the phos-
phorylation potential (2, 3, 7, 28). These beneficial effects are present even during hypoxia (7, 28). However, it is unknown whether the beneficial effects of pyruvate in in vitro systems are present in a whole animal situation.

In the present study, we examined whether intravenous pyruvate could improve vascular, cardiac, and neuronal function during severe hemorrhage and improve survival in swine. To assess metabolic stress during controlled hemorrhage, we measured global indexes of the cytosolic redox state [lactate/pyruvate ratio (L/P)] and indirectly monitored the cytosolic phosphorylation potential of the brain through extracellular (neocortical microdialysis) levels of adenosine and its degradation products, inosine and hypoxanthine. The extracellular accumulation of adenosine purine nucleosides is an indirect indicator of ATP catabolism and decrease in the phosphorylation potential (12, 15).

Microdialysis was chosen because it is minimally invasive and is suitable for repetitive measurements. In the brain, the microdialysis adenosine indexes were also compared with cerebral oxidative metabolism (cerebral oxygen consumption), the net adenosine purine balance compared with cerebral oxidative metabolism (cerebral blood flow, ECOG), and microdialysis instrumentation. After hemodynamic instrumentation, the swine were placed in ventral recumbent position and the cranium was stabilized. The scalp and galea were reflected, and a wide craniectomy was performed. A 6.0-mm ultrasound flow probe (6SB; Transonic Instruments, Ithaca, NY) was placed around the distal sagittal sinus for the measurement of cerebral blood flow (CBF) (14, 24). A 1.5-in. 22-gauge Teflon catheter was inserted into the sagittal sinus to obtain blood samples for cerebrovenous blood gases, purine nucleosides, and L/P measurements. Bilateral gold cup electrodes were placed on the surface of the dura (FD1,2 and CL) for recording of a two-channel bipolar ECOG. The ECOGs were recorded (Aspect 1000, Natick, MA), filtered (1–30 Hz), digitized, and stored on hard disk for review and analysis. Two microdialysis probes (CMA-10; CMA/Microdialysis, Acton, MA) of concentric design (polycarbonate fiber length 4 mm, diameter 0.5 mm, 20,000 Dalton molecular cutoff) were inserted 6 mm into the right frontal neocortex. The location of insertion was 1 cm lateral to the sagittal sinus and 2 and 2.5 cm anterior to the coronal suture. After insertion, the probes were perfused with artificial cerebrospinal fluid (in mM, 155 Na+, 1.1 Ca2+, 0.83 Mg2+, 2.9 K+, pH 7.4) at 2 µl/min using a precision, multisyringe pump (BAS Bee; Bioanalytical Systems, West Lafayette, IN). A micromanometer (MPC-500) was inserted into the subdural space for measurement of intradural pressure. Brain temperature was monitored by an epidural thermistor and maintained at 38.5 ± 0.5°C with heating lamps and a forced-air warming device.

Experimental Protocols

Controlled hemorrhage protocol. One hour before the initiation of hemorrhage, the expired halothane concentration was reduced to 0.5% and the animals were allowed to ventilate spontaneously. Hemodynamic data were recorded every 30 min for the duration of the protocol. Controlled arterial hemorrhage was accomplished by removal of 45% of the estimated blood volume over 15 min (2.0 ml·kg⁻¹·min⁻¹). After the first 15 min, blood was withdrawn as necessary to maintain the MAP at 40 mmHg. With the exception of the protocol infusions of Na+ pyruvate or NaCl, no fluids or shed blood was administered to support the MAP at 40 mmHg. After spontaneous vascular decompensation, death was defined as a MAP <10 mmHg and cessation of spontaneous respiratory effort.

Treatment groups and infusion protocols. The animals were assigned to one of three treatment groups. Group 1 had 30% Na+ pyruvate (pH titrated to 7.4 with 1 N NaOH) infused into the femoral vein 1 h before (infusion A, 1 g·kg⁻¹·h⁻¹) and for 2 h (infusion B, 0.5 g·kg⁻¹·h⁻¹) after the start of hemorrhage. To control for volume and osmolarity effects of the hypertonic Na+ pyruvate, an equivalent volume of 0.9% NaCl (group 2) or Na+ load (10% NaCl, group 3) was infused before (infusion A, 1 h) and after (infusion B, 2 h) the initiation of hemorrhage.

Analytic sampling procedures. Microdialysis samples were continuously collected over 30-min periods (60-µl aliquots) and stored at −80°C until HPLC analysis. One hour after insertion of the microdialysis probes, arterial, mixed venous, and sagittal sinus blood were sampled every 30 min for measurement of pH, base excess, blood gases, hemoglobin, (MPC-650) was inserted through the left femoral artery sheath and advanced into the left ventricle for measurement of left ventricular end-diastolic pressure (LVEDP) and calculation of the first derivative of LV pressure (dP/dt max). The femoral vein was used for infusions. Physiological data were displayed on an eight-channel Hewlett-Packard model 68 clinical monitor.

Cerebral blood flow, ECOG, and microdialysis instrumentation. After hemodynamic instrumentation, the swine were placed in ventral recumbent position and the cranium was stabilized. The scalp and galea were reflected, and a wide craniectomy was performed. A 6.0-mm ultrasound flow probe (6SB; Transonic Instruments, Ithaca, NY) was placed around the distal sagittal sinus for the measurement of cerebral blood flow (CBF) (14, 24). A 1.5-in. 22-gauge Teflon catheter was inserted into the sagittal sinus to obtain blood samples for cerebrovenous blood gases, purine nucleosides, and L/P measurements. Bilateral gold cup electrodes were placed on the surface of the dura (FD1,2 and CL) for recording of a two-channel bipolar ECOG. The ECOGs were recorded (Aspect 1000, Natnick, MA), filtered (1–30 Hz), digitized, and stored on hard disk for review and analysis. Two microdialysis probes (CMA-10; CMA/Microdialysis, Acton, MA) of concentric design (polycarbonate fiber length 4 mm, diameter 0.5 mm, 20,000 Dalton molecular cutoff) were inserted 6 mm into the right frontal neocortex. The location of insertion was 1 cm lateral to the sagittal sinus and 2 and 2.5 cm anterior to the coronal suture. After insertion, the probes were perfused with artificial cerebrospinal fluid (in mM, 155 Na+, 1.1 Ca2+, 0.83 Mg2+, 2.9 K+, pH 7.4) at 2 µl/min using a precision, multisyringe pump (BAS Bee; Bioanalytical Systems, West Lafayette, IN). A micromanometer (MPC-500) was inserted into the subdural space for measurement of intradural pressure. Brain temperature was monitored by an epidural thermistor and maintained at 38.5 ± 0.5°C with heating lamps and a forced-air warming device.

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and oxygen content (IL 1610 and IL 682 CoOx, Instrumentation Laboratories, Lexington, KY). Global oxygen consumption was calculated as \( \text{[(arterial oxygen content mixed - venous oxygen content) cardiac output]} / 100 \). The cerebral metabolic rate for oxygen consumption was calculated as \( \text{[(arterial oxygen content - sagittal sinus oxygen content]} / \text{CBF}] / 100 \). Blood samples for serum electrolytes \( (\text{Na}^+, \text{K}^+, \text{Cl}^-, \text{HCO}_3^-) \), osmolality, lactate, and pyruvate were obtained every 30 min and immediately centrifuged. Blood \((1.5 \text{ ml arterial, mixed venous, sagittal sinus})\) for purine analysis was collected with 1.5-ml ice-cold dipyridamole \((400 \mu\text{M})\) to minimize purine uptake and metabolism by cellular blood components. The dipyridamole-treated samples were immediately centrifuged and 0.5 ml of the supernatant was mixed with 0.5 ml 6 N \( \text{HClO}_4 \) on ice to denature and precipitate plasma proteins. Deproteinization was followed by immediate neutralization with \( \text{KHCO}_3 \) to a slightly acidic \( \text{pH} \) of 6.0–7.0. After standing on ice for 30 min, the \( \text{KClO}_4 \) precipitate was removed by centrifugation. Samples were stored at −80°C until HPLC analysis.

**Analytic Methods**

HPLC sample preparation and analysis. Analysis of hypoxanthine \((\text{HX})\), inosine \((\text{Ino})\), and adenosine \((\text{ADO})\) in the dialysate and blood was performed using a multiwavelength reverse-phase HPLC technique. Neocortical microdialysis samples \((30 \mu\text{l})\) were injected directly into a C-18 column. Sample aliquots \((10 \mu\text{l})\) were injected into the HPLC workstation using a refrigerated automated injector system \((\text{Waters Wisp 712; Waters Associates, Milford, MA})\). Peak identification and quantification were accomplished by comparing known retention times with peak absorbance ratios at four different wavelengths \((254, 263, 273, 293 \text{ nm})\) using a programmable multi-wavelength detector \((\text{model 490, Waters Associates})\). Chromatograms and absorbance data were recorded and analyzed using the Waters Maxima software \((\text{V 6.11, Waters Associates})\).

Microdialysis calibration procedures. In vitro recovery for purines for each microdialysis probe was determined in triplicate before and after each experiment. The probes were immersed in a purine calibration standard solution at 38.5°C and perfused with artificial cerebrospinal fluid. The calibration standards contained \( \text{HX}, \text{Ino}, \text{and ADO dissolved in double-distilled deionized water at a final concentration of } 10 \mu\text{M for each compound. The HPLC peak heights of the calibration standards contained } \text{HX, Ino, and ADO dissolved in double-distilled deionized water at a final concentration of } 0.9\% \text{ NaCl (volume control), and } 10\% \text{ NaCl (osmotic control groups were similar (44.9 ± 1.1, 45.2 ± 0.8, and 45.1 ± 1.1 kg, respectively). The intravenous pyruvate infusion raised the arterial pyruvate concentration from 0.12 ± 0.01 to 5.68 ± 0.91 mM 30 min after the start of the protocol infusion (Fig. 1) and this concentration was maintained during the infusion. After cessation of the pyruvate infusion, the concentration decreased to 6.25 ± 0.73 to 1.02 ± 0.14 mM within 30 min. In both the volume and osmotic control groups, the prehemorrhage pyruvate levels ranged from 0.08 to 0.18 mM and increased to 0.32 ± 0.03 and 0.33 ± 0.04 mM, respectively, during hemorrhage (Fig. 1).}

Intravenous Pyruvate Effects on Hemodynamics, Oxygen Delivery, and Oxygen Consumption

There were no significant differences in the initial global hemodynamics between the groups (Table 1). However, 1 h after the start of the protocol infusion both the pyruvate and osmotic control groups had a median frequency and amplitude from the digitized ECOG were averaged for the last 5 min of each measurement period.

Data Presentation and Statistics

All data are presented as means ± SE or median with range in text and tables. The means ± SE are shown in the figures. Differences between groups for nonrecurring measurements were assessed using ANOVA. Analysis of differences between and within groups for repeated measurements was performed using ANOVA between groups for the dependent variable \((\text{i.e., MAP, CBF, etc.) with repeated measures over time. Within and between group testing was accompanied by a Tukey honestly significant difference multiple-range test to correct for multiple comparisons. Nonparametric analysis was performed on the ECOG data, which was ordinal, or data that did not approximate a normal distribution. Values were considered statistically different at \( P < 0.05 \) after correction for multiple comparisons.

**RESULTS**

The animal weights in the pyruvate, 0.9% NaCl (volume control), and 10% NaCl (osmotic control) groups were similar \((44.9 ± 1.1, 45.2 ± 0.8, and 45.1 ± 1.1 kg, respectively). The intravenous pyruvate infusion raised the arterial pyruvate concentration from 0.12 ± 0.01 to 5.68 ± 0.91 mM 30 min after starting the infusion (Fig. 1) and this concentration was maintained during the infusion. After cessation of the pyruvate infusion, the concentration decreased from 6.25 ± 0.73 to 1.02 ± 0.14 mM within 30 min. In both the volume and osmotic control groups, the prehemorrhage pyruvate levels ranged from 0.08 to 0.18 mM and increased to 0.32 ± 0.03 and 0.33 ± 0.04 mM, respectively, during hemorrhage (Fig. 1).

![Fig. 1. Arterial pyruvate levels (means ± SE) before and during controlled hemorrhage](image-url)
significant increase in the cardiac index (CI) and stroke volume (Table 1, H30). In the pyruvate group, the enhanced cardiac performance was associated with a 25% rise in the left ventricular dP/dtmax with no change in heart rate, LVEDP, or MAP. With the enhanced inotropic state in the pyruvate group, the increases in CI and stroke volume in the osmotic control group occurred with an increase in the LVEDP and no increase in contractility (dP/dtmax). Thus the pyruvate and not the osmotic or volume treatments enhanced the contractile state of the left ventricle during the prehemorrhage phase.

During the hemorrhage phase, the CI was higher in the pyruvate group at H30 (Table 1) with no differences between the groups at H60 (Table 1). During the first hour of hemorrhage (H30 and H60), there were no differences between groups in the MAP. At H30, the MAP in the volume and osmotic control groups was 14 and 20% lower, respectively, than in the pyruvate group, indicating the onset of vascular decompensation. At H120 the control groups had no measurable MAP, whereas the pyruvate-treated animals still maintained a MAP >30 mmHg. After the pyruvate infusion was stopped at H120, the pyruvate animals showed a rapid fall in MAP, CI, and dP/dtmax. The average time until cardiovascular decompensation and death in the pyruvate-treated group was 151.2 ± 10.0 min, whereas the time to death was 82.7 ± 5.5 and 74.8 ± 8.2 min in the volume and osmotic control groups, respectively (P < 0.001). The prolonged survival in the pyruvate group was not secondary to differences in volume status or protocol management because the peak hemorrhage volumes (44.9 ± 1.6, 41.8 ± 1.1, and 43.2 ± 1.5 ml/kg) and the times to reach the peak hemorrhage volume (59 ± 4, 55 ± 4, and 58 ± 5 min) were similar in the pyruvate, volume control, and osmotic control groups, respectively.

Table 2 shows the systemic and cerebral oxygen delivery and consumption data. Before hemorrhage (H0), there was an increase in systemic oxygen delivery in the pyruvate and osmotic control groups, which was secondary to the augmented CI (Table 1, H0). There were no differences between groups for the other parameters. At H30 and H60, systemic oxygen consumption was 40 and 30% higher in the pyruvate group compared with the control groups. In addition, despite the comparable decreases in the cerebral perfusion pressure (Table 1), the pyruvate group maintained the cerebral oxygen consumption 75% higher than the controls at H30, H60, and H90.
Table 2. Systemic and cerebral oxygen delivery and consumption measurements

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<th>Variable</th>
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<th>Initial</th>
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<th>H60</th>
<th>H90</th>
<th>H120</th>
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<td>CBF, ml·100 g⁻¹·min⁻¹</td>
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<td>65.9±4.5</td>
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</table>

Values are means ± SE. Initial measurements were made before start of pyruvate or control infusions. H₂ was 1 h after start of infusions and immediately before initiation of hemorrhage (H). Measurements during hemorrhage were performed every 30 min. DO₂, systemic oxygen delivery; VO₂, systemic oxygen consumption; CBF, cerebral blood flow; CDO₂, cerebral oxygen delivery; CMRO₂, cerebral oxygen consumption. *P < 0.05 compared with pyruvate group for time-matched data.

Intravenous Pyruvate Effects On Blood Electrolytes, Acid-Base Status, Lactate, and L/P

The acid-base status, lactate and pyruvate levels, and the L/P concentration ratios are shown in Fig. 2. Serum bicarbonate data are shown in Table 3. Before hemorrhage, the pyruvate infusion caused an increase in the pH (Fig. 2A), base excess (Fig. 2B), and serum bicarbonate (Table 3) to 7.54, 12 meq/l, and 38 meq/l, respectively. During hemorrhage, the control groups developed a substantial metabolic acidosis with a fall in arterial pH to 7.21, base excess to −10 meq/l, and serum bicarbonate to 17 meq/l at H₉₀. In contrast, the pyruvate infusion completely prevented the development of a metabolic acidosis with the pH, base excess, and serum bicarbonate returning to the basal levels only after 150 min of hemorrhage.

The initial lactate levels (1.2 ± 0.1 mM) and L/P ratios (7–15) were within the physiological range for all animals (Fig. 2, C and D). In the control groups, the lactate levels and the L/P ratios were unchanged before hemorrhage and increased progressively to near 12 mM and 40 during hemorrhage (H₉₀). In contrast, the pyruvate infusion before hemorrhage caused an increase in lactate to 8.6 ± 0.8 mM and a decrease in L/P to 1.0 ± 0.2. During hemorrhage the lactate concentration increased to 30–40 mM, whereas the L/P rose to only 5.6 (Fig. 2, C and D). After termination of the pyruvate infusion at H₁₂₀, the L/P rose to the peak levels.
In the pyruvate group there was no significant increase in the microdialysis purines at 30 min. At 60 min, the values were greater than the basal values but still significantly lower than in the control groups. At 90 min, the microdialysis purine levels were not different.

The microdialysis probe recoveries using the standard solution of ADO, Ino, and HX were 29.0 ± 0.7, 26.5 ± 0.8, and 31.5 ± 0.9%, respectively, with no differences between the experimental groups. The microdialysis samples collected immediately after probe insertion showed composite purine levels (ADO + Ino + HX) of 100–120 µM, which decreased to 20 µM before the start of hemorrhage. During hemorrhage, the microdialysis purine levels in the control groups rose rapidly, peaking at 90 µM at 90 min (Fig. 3A). In the pyruvate group there was no significant increase in the microdialysis purines at 30 min. At 60 min, the values were greater than the basal values but still significantly lower than in the control groups. At 90 min, the microdialysis purine levels were not different.
in the three groups. Figure 3 also shows that while the accumulation of microdialysis purines were comparable in all groups at 90 min, the pyruvate-treated animals did not exhibit a net release of purines into the sagittal sinus (Fig. 3B). These data in conjunction with the improved cerebral oxygen utilization (Table 2) suggest that pyruvate enhanced neocortical metabolic function and stability during hemorrhagic shock.

**Intravenous Pyruvate Effects on ECOG**

The ECOG data are summarized in Table 4. During hemorrhage, slowing of the ECOG and loss of amplitude were observed in the visual evaluation, median frequency, and amplitude of the ECOG. In all groups, the loss of high-frequency activity occurred before significant decreases in the amplitude. The beneficial effects of pyruvate were evident in the higher visual evaluation scores and delay in the frequency and amplitude losses compared with the time-matched data from the control groups. A representative series of 5-s ECOG tracings from a pyruvate and a control animal are shown in Fig. 4. In the tracings from the control animal, there was slowing of the frequency 30 min after the start of hemorrhage and further slowing of frequency and loss of amplitude at 60 min. The tracing from 90 min after the initiation of hemorrhage was essentially isoelectric. In contrast, the ECOG from the pyruvate animal showed slowing of the frequency and loss of amplitude only at 120 and 150 min, respectively, and an isoelectric tracing at 180 min after the start of hemorrhage (60 min after stopping intravenous pyruvate).

**DISCUSSION**

Acute hemorrhage causes a shock state characterized by hypotension caused by a decreased intravascular volume and cardiac output that is compensated for by the release of endogenous vasoconstrictors and the recruitment of extravascular volume. Severe prolonged hemorrhage, however, initiates a sequence of events that ultimately results in refractory vascular failure, cardiovascular decompensation, and death. The specific aim of this investigation was to determine if the infusion of a metabolic substrate, pyruvate, could improve survival during hemorrhagic shock.
prove functional and metabolic status and delay the time until death during severe hemorrhagic shock in swine. We used an isobaric arterial hemorrhage model (40 mmHg) to induce the acute loss of approximately 66% of the estimated blood volume. With the exception of the experimental (30% pyruvate) and control (0.9% or 10% NaCl) infusions, cardiovascular decompensation was not treated and resulted in death. The shock state induced in the control animals was characterized by a substantial metabolic acidosis (pH 7.2, HCO₃⁻ 17 meq/l), accumulation of lactate (12 mM), an increase in the L/P (40), hyperkalemia (7–8 meq/l), and death within 90 min. However, in animals treated with pyruvate, the time to cardiovascular collapse and rise in the specific indicators of cellular dysfunction and metabolic decompensation were delayed by 75 min. Whereas this highly instrumented swine model does not represent an uncontrolled hemorrhage scenario, it did allow for the delineation of the effects of pyruvate on metabolic and functional status during global and neocortical delivery dependent oxygen consumption. Furthermore, it was feasible to evaluate the neocortical energetic status qualitatively using the phosphorylation potential-related purine nucleoside parameters.

Cardiovascular Effects on Pyruvate During Controlled Arterial Hemorrhage

Pyruvate, a key intermediate of oxidative metabolism, has been demonstrated to exert positive inotropic effects on the heart and enhance myocardial energetic stability in vivo and in vitro (4, 7, 21, 22, 28, 34). Those effects are consistent with the observed enhancement of the inotropic state of the myocardium before hemorrhage. However, the interpretation of the effects of pyruvate on cardiovascular function during the hemorrhage phase is more complex. In this investigation, the most noteworthy effect of pyruvate was the 75-min prolongation in survival. Another contribution to the higher oxygen delivery and consumption during the initial 30 min of hemorrhage can completely account for the 75-min prolongation in survival. The shock state induced in the control animals was characterized by a substantial metabolic acidosis (pH 7.2, HCO₃⁻ 17 meq/l), accumulation of lactate (12 mM), an increase in the L/P (40), hyperkalemia (7–8 meq/l), and death within 90 min. However, in animals treated with pyruvate, the time to cardiovascular collapse and rise in the specific indicators of cellular dysfunction and metabolic decompensation were delayed by 75 min. Whereas this highly instrumented swine model does not represent an uncontrolled hemorrhage scenario, it did allow for the delineation of the effects of pyruvate on metabolic and functional status during global and neocortical delivery dependent oxygen consumption. Furthermore, it was feasible to evaluate the neocortical energetic status qualitatively using the phosphorylation potential-related purine nucleoside parameters.

Another important effect of pyruvate in extending short-term survival was the obvious delay in the onset of vascular failure. Pyruvate produced a 30-min delay from peak hemorrhage volume until spontaneous decline in the MAP. Possible explanations for this pyruvate-induced maintenance of vascular performance are the absence of a metabolic acidosis, stabilization of the cytoplasmic redox potential (L/P), and the known beneficial effects of pyruvate on oxidative metabolism and the phosphorylation potential during metabolic stress.

In VSM, decreases in pH and the cellular phosphorylation potential are known to cause K⁺-channel activation with a decrease in intracellular Ca²⁺ concentration and contractility (1, 25). K⁺-channel activation has been shown to be a major component in the vascular insensitivity to α-adrenergic agonists, during both hemorrhagic and endotoxic shock (27, 33).

Although direct evidence supporting differences in K⁺-channel activation or intracellular Ca²⁺ concentration caused by pyruvate is not provided by this investigation, the absence of hyperkalemia in the pyruvate group is consistent with reduced K⁺-channel activity. Mechanistically, pyruvate may have attenuated K⁺-channel activation by minimizing the development of acidosis and depletion of ATP in VSM. Whereas pyruvate, as much as lactate, causes intracellular acidosis when applied to isolated incubation systems (10), we observed systemic alkalinization in the whole animal and no acidosis during shock. The prevention of acidosis could be caused by the cytoplasmic reduction of pyruvate to lactate with a net decrease of two hydrogen ions. Additionally, pyruvate import into the mitochondria requires H⁺ co-transport and the hydrogen ion is removed when pyruvate is oxidized to carbon dioxide and water during respiration. In addition to alkalinization, pyruvate enhances the thermodynamic state of ATP, stimulates anaplerosis of the citric acid cycle, and increases mitochondrial NADH and oxygen consumption (2, 3). In VSM, glycolytic ATP production is the primary source of energy for maintenance of transsarcolemmal ion gradients (35). Nevertheless, pyruvate, a direct oxidative metabolic substrate, can also maintain normal ionic gradients, Ca²⁺ flux, and vascular contractility even in the absence of glycolysis (2, 3, 20). Thus the prevention of acidosis in conjunction with the stimulatory effects of pyruvate on oxidative metabolism and cellular energetics may have prevented or attenuated K⁺-channel activation and delayed the onset of a hypocontractile state.

A third possible beneficial effect of exogenous pyruvate on VSM function may be related to changes in the cytoplasmic redox potential as indicated by the arterial L/P. In contracting porcine carotid arteries, 10 mM lactate induces changes in the cytoplasmic redox state (L/P 68) that are associated with a 30% decrease in sustained KCl contraction compared with pyruvate (10 mM) (2). If this applies in vivo, then cytoplasmic redox changes induced by pyruvate (L/P < 6) during hemor-
Hemorrhagic shock, compared with controls (L/P > 30), may have helped to maintain the contractile state of the vascular system.

Neocortical Effects of Pyruvate During Hemorrhagic Shock

Because of its high metabolic rate, limited energy stores, and nearly complete dependence on oxidative metabolism for ATP generation, the brain requires a constant supply of oxygen and oxidative substrate for normal function. To conserve energy stores and efficiently distribute substrate, blood flow is coupled to and distributed on the basis of the metabolic demands of electrophysiological function and cellular homeostasis. During severe reductions in substrate delivery, electrophysiological function (synaptic transmission) is sacrificed to conserve energy for the homeostatic metabolic demands necessary to maintain ion gradients and the membrane potential. During ischemia, an increase in the extracellular adenosine purines occurs with a 50% reduction in CBF (23), which is before the onset of severe ischemic changes in the electroencephalogram (29). In addition, adenosine purine nucleoside changes occur reciprocally with the phosphorylation potential (6, 16, 17) and ATP pool size (15). The microdialysis purine levels are also a more sensitive marker of ischemic CBF than excitatory amino acid release in both cats (23) and humans (12). Furthermore, both electrophysiological amplitude and frequency reductions, as well as the microdialysis accumulation of hypoxanthine, correlate with ischemia and are predictive of poor clinical outcome in humans (12).

Before hemorrhage in the current investigation, pyruvate administration had no measurable effects on CBF, the ECOG, or the microdialysis adenosine purine levels (H0, Tables 2 and 4, Fig. 3A). However, during hemorrhagic shock the pyruvate-treated animals showed definite evidence of improved cerebral metabolic and electrophysiological function (Tables 2 and 4, Fig. 3). The enhanced neocortical metabolic function and stability were demonstrated by 1) delayed and reduced early accumulation of microdialysis purines (Fig 3A), 2) lack of net purine release into the sagittal sinus throughout the hemorrhage phase (Fig 3B), and 3) maintenance of a normal ECOG for more than 60 min of hemorrhage (Table 4). In this study, pyruvate delayed (30 min) and markedly attenuated (60 min) the rise in the microdialysis purine accumulation. Although there were no differences in the purine levels between the pyruvate and control groups at 90 min, the ECOG was only moderately depressed compared with severe depression or complete absence of ECOG activity in the controls. Under the same conditions, pyruvate prevented the net release in purines into the sagittal sinus from the neocortex. These observations indicate that the local neuronal ATP in the immediate vicinity of the microdialysis probes was degraded and the cells de-energized at 90 min in all groups. However, the lack of net efflux into the sagittal sinus indicates that pyruvate preserved or stabilized the global neocortical ATP pool. In addition, the global improvement in the energy state and ischemic tolerance in the pyruvate group are consistent with the prolonged maintenance of the ECOG (Table 2 and Fig. 4) (30). In the pyruvate group, the improved energetic and functional parameters occurred in conjunction with a significantly higher CBF and cerebral oxygen consumption at similar perfusion pressures. Because CBF and cerebral oxygen consumption are coupled to cerebral metabolic demand, the pyruvate enhancement of the metabolic status appears to be responsible for these differences. This may represent improved CBF autoregulation at these low perfusion pressures or perhaps a redistribution of CBF and substrate within the brain. This interpretation is speculative because regional CBF was not measured.

In this study the measured indicators of cerebral ATP, energetics, and ECOG function provide evidence that pyruvate enhances cerebral ischemic tolerance during severe hemorrhagic shock. Previous studies have shown that millimolar pyruvate protects cerebral striatal cells from hydrogen peroxide toxicity and glutamate-mediated neurotoxicity in respiring hippocampal slices (11, 18). In vivo studies of partial or complete cerebral ischemia show that metabolic support through pyruvate dehydrogenase activation with dichloroacetate or acetylcarnitine normalizes postischemic levels of ATP and phosphocreatine, improves the clearance of intracellular acidosis, and substantially improves neurological outcomes (8, 26). However, pyruvate dehydrogenase activation alone does not prolong survival during hemorrhagic shock (31). Thus this is the first whole animal study to show that metabolic substrate administration enhances neuronal ischemic tolerance and prolongs survival during the ischemic conditions of hemorrhagic shock.

Limitations of Study

There are three limitations of this study. The first is that the dose response for the beneficial effects of pyruvate remains unknown. In this study, the decision to target 5 mM pyruvate was based on previous work in isolated perfused hearts (7, 21, 28), VSM (2), and neuronal cell culture experiments (11, 18), which all show beneficial functional and/or metabolic effects of 2–10 mM pyruvate. Because only one concentration was evaluated in this study, the minimum effective concentration was not established. Another limitation is that the pyruvate was administered before and not after the development of the shock state. Although this design revealed beneficial effects of pyruvate during both the basal normoxic state as well as during ischemia, additional studies are needed to determine the efficacy of pyruvate following the development of shock.

The administration of the Na+ salt of pyruvate in this study also caused biochemical and acid-base changes. These were the increase in serum lactate, the metabolic alkalosis, and the increases in osmolality and serum Na+. Normally, substantial lactate accumulations would be a major concern because, in conjunction with a high L/P and metabolic acidosis, it is a marker of cellular hypoxia and anaerobic glycolysis. However, in this investigation the high lactate level was driven by
exogenous pyruvate and was associated with an L/P below basal levels and the prevention of a metabolic acidosis despite severe hemorrhagic shock (Fig. 2). The other biochemical changes were the increases in osmolality and Na\(^+\). These changes were secondary to the hypertonic 30% Na\(^+\)-pyruvate formulation and substituting part of the Na\(^+\) content with other cations (Ca\(^{2+}\), choline, or ammonium) or a metabolizable pyruvate ester could minimize these effects.

Another limitation is that evidence in support of the metabolic mechanisms underlying the beneficial effects of pyruvate is indirect, because the actual phosphorylation potential, the cytosolic redox state, and the intracellular pH were not directly measured. However, other studies have established that an increase in extracellular purine nucleosides results from decreases in the cytosolic phosphorylation potential or energy status in the heart (6, 16). In addition, there are firmly established effects of millimolar pyruvate on the cytosolic redox status, cellular anaplerosis, improved oxygen utilization, and increases in the phosphorylation potential in intact myocardium (4, 7, 21, 28) and VSM (2).

In conclusion, the administration of intravenous pyruvate before and during hemorrhagic shock may have a major therapeutic potential for the delay, if not prevention, of global and cerebral metabolic failure, cardiovascular decompensation, and death. Our data show that these benefits are not related to volume or osmotic effects. Currently, widespread application of the principle of metabolic support during shock states is hampered by the incomplete understanding of the mechanism(s) by which pyruvate exerts its beneficial effects in the whole animal. Also, the minimum effective dose needs to be defined. Future work will determine if pyruvate will be useful only as a prophylactic agent or also as part of a resuscitative regimen.

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