**Title and Subtitle**
Cumulative Effect of Repeated Brief Cerebral Ischemia

**Funding Numbers**
- F49620-92-J-0362
- 61102F
- 2312
- BS

**Performing Organization Name(s) and Address(es)**
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**Performing Organization Report Number**
- AFOSR-TR-88-039

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**Supplementary Notes**
Approved for public release; distribution unlimited

**Abstract**
The purpose of this grant is to investigate the metabolic and physiological factors determining the extent of ischemic damage in a model of brief repetitive ischemia. The model is intended to simulate the effects of Gz induced blackout experienced by fighter pilots undergoing high gravitational stress maneuvers. To achieve this goal a rat model was developed whereby ischemia could be induced remotely (under computer regulation) by inflation of occlusive cuff about the common carotid artery of the rat. Metabolic parameters were determined by performing in vivo NMR measurements throughout the repetitive ischemic insult. The non-destructive nature of the NMR measurement permits complete time courses to be monitored in each animal thereby minimizing animal variability. NMR measurements of metabolite content are then used in conjunction with histologic evaluations of neuronal death to assess which metabolic factors have the strongest influence on ischemic damage. During the past year we have completed our studies: a) investigating the correlations between EEG, phosphocreatine (PCr), adenosine triphosphate (ATP), inorganic phosphate (P), pH and lactate as a function of increasing ischemic severity in our model of brief

**Subject Terms**

**Number of Pages**

**Price Code**

**Security Classification of Report**
(U)

**Security Classification of This Page**
(U)

**Security Classification of Abstract**
(U)

**Limitation of Abstract**
(U/L)
A. Publication in Reviewed Journals:


Submitted:


In Preparation:


B. Books or Book Chapters Published

None.

C. Graduate Students

None.

D. Post Doctorates

Tan, Min-Jie, Neurology non-US
Lou, Kang-Li, Neurology non-US

E. Awards

None.
I INTRODUCTION

The purpose of this grant is to investigate the metabolic and physiological factors determining the extent of ischemic damage in a model of brief repetitive ischemia. The model is intended to simulate the effects of Gz induced blackout experienced by fighter pilots undergoing high gravitational stress maneuvers. To achieve this goal a rat model was developed whereby ischemia could be induced remotely (under computer regulation) by inflation of occlusive cuff about the common carotid artery of the rat. Metabolic parameters were determined by performing in vivo NMR measurements throughout the repetitive ischemic insult. The non-destructive nature of the NMR measurement permits complete time courses to be monitored in each animal thereby minimizing animal variability. NMR measurements of metabolite content are then used in conjunction with histologic evaluations of neuronal death to assess which metabolic factors have the strongest influence on ischemic damage.

During the past year we have completed our studies: a) investigating the correlations between EEG, phosphocreatine (PCr), adenosine triphosphate (ATP), inorganic phosphate (P), pH and lactate as a function of increasing ischemic severity in our model of brief repetitive ischemia and b) characterizing substrate utilization and lactate clearance curves using the same model. These experiments have been performed in the rat brain using the repetitive ischemia model with in vivo NMR to measure metabolite content. We are expanding these studies to assess the issue of selective vulnerability by correlating the regional time course of lactate elevations (5 minute time resolution) with post mortem histologic evaluation. These studies will be carried out over the next year and will provide a link between the metabolic alterations seen in this model and cellular damage.
II PROGRESS

The first two phases of our work, evaluation of the serial changes in PCr, ATP, pH, P, and lactate during the repetitive ischemia and the characterization of substrate utilization and lactate clearance have been completed. The work has been reported in three abstracts (1-3) and is described in two manuscripts (4,5) which are being submitted for publication in the Journal of Cerebral Blood Flow and Metabolism (see attached copies). The results of this work is presented in the following sections.

A. Time Course of Metabolite Changes during Repetitive Ischemia

1. Introduction: The goal of this work was to evaluate the temporal progression of the metabolic and functional changes accompanying repetitive brief cerebral ischemic episodes. To evaluate the time course of the metabolic changes serial interleaved \(^1\)H and \(^{31}\)P NMR spectra were collected while rats were subjected to 30 one minute episodes of cerebral ischemia and reflow followed by thirty minutes of continuous reflow. To evaluate the effect of the repeated ischemic episodes on brain function, fast and slow components of the EEG were monitored throughout the ischemic and reflow periods. The severity of the ischemia was modulated by altering the ischemic duration during the one minute period.

2. Methods: Male Sprague Dawley rats (200-240gm) were anesthetized with 70% \(\text{N}_2\text{O}\), 29% \(\text{O}_2\), and 1% Halothane, paralyzed with tubocurarine chloride and mechanically ventilated. The external carotid and both subclavian arteries were ligated. The right common carotid artery was bi-directionally cannulated for monitoring the carotid stump pressure (reflecting the cerebral perfusion pressure at the Circle of Willis) and for measurement of systemic pressure (figure 1). Rectal temperature was monitored throughout the experiment and maintained at \(37^\circ\)C by use of feedback regulated heating chamber surrounding the animal. Ischemia was induced by an inflatable occlusive cuff placed about the left common carotid artery. Inflation and deflation of the cuff was controlled by a hydraulic system linked to a IBM AT computer. EEG activity was monitored by placement of two silver wire electrodes. The resultant signal was analog filtered to obtain fast (7-20Hz) and slow (2-5Hz) components and digitized every half second throughout the experiment.

Each experiment consisted of: 1) an initial period of 10 minutes for control measurements; 2) followed by 30 minutes of 30 episodes of brief cerebral ischemia and reflow; 3) concluding with 30 minutes of continuous reflow. The severity of the ischemia was modulated by altering the durations of the ischemic and non-ischemic durations during the one minute interval. Four protocol were utilized in 20 studies: 101:50R, 10 seconds of ischemia followed by 50 seconds of reflow \(n=6\), 201:40R \(n=6\), 301:30R \(n=4\) and 401:20R \(n=4\).

NMR data was collected using a 4.7T 40cm bore Bruker Biospec system using a two coil \(^1\)H/\(^{31}\)P detector. An elliptical \(^{31}\)P surface coil (8x12mm) was placed directly upon the exposed skull. The \(^1\)H coil, was placed immediately above the \(^{31}\)P coil and consisted of two 10mm circular loops connected in a butterfly configuration. The butterfly configuration minimizes coupling between the coils. To minimize the acquired lipid signal, the scalp was removed and the temporal muscles retracted. \(^1\)H and \(^{31}\)P spectra were acquired simultaneously by interleaving \(^1\)H and \(^{31}\)P acquisitions on a scan by scan basis. The data was acquired in one minute bins, (60 scans at a 1 second repetition time for both nuclei). \(^{31}\)P spectra were summed in a moving
average of four adjacent one minute measurements to improve the S/N. Resonance areas were
determined by peak integration. \(^1\)H resonance areas were converted to millimolar values using
a 11mM value for cerebral creatine as an internal standard, and corrected for NMR measurement
parameters.

3. Results:

i. Ischemia Model: The computer controlled occluder system provided a highly
reproducible ischemic insult with minimal occlusion to occlusion variations in the carotid stump
pressure during each subsequent ischemic episode. Typical systemic and carotid stump
pressures during the 4 ischemic protocols are displayed in figure 2. The carotid stump pressure
during the ischemic period was typically 7-10mM with variations of approximately 1-2mM seen
for a given animal.

ii. EEG Activity: Both the slow and fast components were seen to decline during the
ischemic interval of each ischemia/reflow episode in the 201:40R, 301:30R and 401:20R animals
(figure 3). Minimal changes were seen in the 10:50R animals, most likely due to the shortness
of the interval and the finite time required for cuff inflation and consumption of brain oxygen
stores. To facilitate the analysis of long term trends, the EEG data was averaged over the one
minute ischemia/reflow episode and for all animals in each group (figure 4). In the 10:50R
animals the average fast activity was unchanged and a mild increase in slow activity was seen.
Fast and slow activity was seen to decline in the 201:40R, 301:30R, and 401:20R protocols
achieving an average decline of greater than 80% in fast activity in the 401:20R protocol. The
201:40R animals showed an initial rapid decline in EEG until reaching an apparent steady state
at approximately 60% of control period fast activity. Slow activity in these animals was also found
to decline but a mild recovery was subsequently seen. In the 301:30R and 401:20R animals, the
depression in average EEG activity was found to be progressive with the depth of the depression in
each period increasing with each successive ischemic episode. This trend continued until the
EEG was virtually gone in the 401:20R animals. Upon reflow all groups showed a recovery of fast
and slow activity reaching levels near or above control values by the end of thirty minute reflow
period.

Therefore, the 10:50R protocol appears to establish a threshold for ischemic duration in
this model below which significant changes in fast EEG activity do not occur. This minimum
interval most likely reflects the amount of time required to establish the ischemia (several
seconds for balloon inflation) and deplete brain oxygen stores (6). The 20:40R animals establish
an apparent steady state of decreased EEG, such that the effects of each ischemic episode on
EEG are balanced by each reflow period. In contrast, the 30:30R and 40:20R animals show an
incremental decline throughout the thirty episodes of ischemia in EEG activity reflecting the
inability of the brain to compensate for the cumulative effects of each new ischemic period.
Specifically, the amount of fast EEG activity during each reflow period was found to decline. Thus
the 30:30R animals set a lower threshold for a slow progressive decline in EEG activity.

iii. ATP, PCr and Pi: Displayed in figure 5 are representative \(^3\)P spectra from a 401:20R
animal: during control, at the conclusion of the thirty episodes of ischemia and after thirty
minutes of reflow. Similar to the EEG, the 10:50 ischemic protocol also did not result in a
significant decrease in PCr when the \(^3\)P data was averaged over 4 consecutive ischemia/reflow
episodes (figure 6). However significant declines in PCr content relative to the control period
were observed in the other groups. As expected the longer ischemic intervals resulted in greater
decline in PCr, with the 401:20R protocol resulting in a 75% decrease in PCr. The PCr time
course data is qualitatively similar to that of the EEG. The 20I:40R animals showed a rapid decline followed by a plateau region (75% of control) whereas the 30I:30R and 40I:20R animals showed rapid decreases followed by slow progressive declines. All animals showed near complete PCr recovery at the end of the thirty minutes of continuous reflow.

Significant changes in average ATP were visualized only in the 40I:20R animals. This is consistent with a model where ATP is preserved at the expense of PCr. Therefore the 50% average decrease in PCr seen in the 30I:30R animals does not appear to be sufficient to induce a significant change in the average ATP content, despite substantial changes in the EEG. This effect may reflect a strong link between PCr and EEG activity or possibly reflect the averaging nature of the acquisition. Specifically, short term reductions in ATP during each ischemic interval may occur which might not appear as significant decreases in the 4 minute ischemia/reflow average data.

Cerebral P, levels are reported as a fraction of the pre ischemia PCr content. Similar to that seen in the ATP data a significant increase in P, content is visualized only in the 40I:20R animals, where substantial ATP hydrolysis augments the PCr dephosphorylation. However, the change in the 40I:20R group is substantial, displaying an increase of greater than 3 fold.

iv. Lactate and pH: Displayed in figure 7 are one minute spectra acquired during the control, at the conclusion of ischemia and at the conclusion of reflow in a 40I:20R animal. The resonance at 1.33 ppm has contributions from extracerebral lipids, lactate, threonine and alanine and lactate. To minimize the effects of these contaminants, the lactate data is reported as a difference in the 1.33 ppm resonance area from the average control value. Since contributions from extracerebral lipids should not be changing during the experiment and changes in lactate in millimolar quantities should dominate the changes in alanine and threonine, the difference data should provide a good estimate of the lactate accumulation induced by the ischemia. Displayed in figure 8 are average time courses of the lactate changes for the four groups of animals. In this study lactate proved to be the most sensitive indicator of ischemia, showing significant elevations in all four groups. Similar to the changes in EEG, lactate levels in the 20I:40R group showed a rapid increase to a plateau level. The plateau level resulting from a complete balance between clearance and generation rates. Lactate changes in 30I:30R and 40I:20R groups again were qualitatively similar to the EEG changes showing rapid initial elevations followed by slow progressive increase. Despite the large changes in lactate observed in 40I:20R animals, approximately 18mM, lactate clearance during the subsequent reflow period was complete, with all animals returning to near control levels.

The time course of pH changes were qualitatively similar to that seen in the lactate data. The observed pH changes ranged from initial levels of 7.15 in the control period to 7.1 at the conclusion of the ischemic episodes in the 10I:50R group to 7.0, 6.8 and 6.6 in the 20I:40R, 30I:30R and 40I:20R animals respectively. Again all animals showed complete recovery to control values during the reflow period. Correlation of the pH and lactate data revealed a general trend towards decreasing pH with increasing lactate. However, the data was found to segregate according to duration of ischemia with decreased pH being associated with increased ischemic duration despite identical lactate levels.

vi. EEG - Metabolic Correlations: Significant changes in lactate, pH and PCr content were found in all animals that approximately paralleled the decreases in EEG activity. To evaluate the relationship between these metabolic (lactate, pH and PCr) and functional variables (fast and slow components of the EEG), EEG was plotted as a function of lactate, pH and PCr (figure 9).
Although all three variables showed strong with trends with fast activity, the relationship between lactate activity was different depending on the ischemic group viewed. Specifically, greater declines in fast activity were observed for the same increase in lactate in the longer duration ischemias. Excellent correlations were seen between the pH and PCr levels in the 20:40R, 30:30R and 40:20R animals.

4. Summary: We have demonstrated that brief repetitive ischemias can produce progressive highly reproducible declines in PCr, pH and increases in lactate. The severity of these changes is easily modulated by altering the ratio of the ischemic to reflow period during each episode. EEG was also shown to decline in this model and be most strongly correlated with changes in PCr and pH. Lactate changes were found to parallel the changes in EEG, however the level of lactate accumulation was found to vary substantially for the same decrease in EEG. Specifically, larger declines in average EEG were seen at equivalent lactate levels for animals subjected to longer ischemic periods. The PCr and EEG data are consistent with a model whereby the amount of fast activity is directly proportional to the availability of PCr. The strong relationship between pH and EEG is most likely influenced by the creatine kinase equilibrium, and therefore at least partially reflects the availability of PCr. Finally, the ischemia appears to be fully reversible, since all metabolic and functional correlates returned to control levels. This complete metabolic recovery may permit each animal to serve as its own control in determining the metabolic effect of different therapeutic interventions. However, histologic evaluations were not performed on these animals, such that the presence of cellular damage can not be excluded in the more severe protocols. Studies investigating differences in regional metabolic sensitivity and histologic correlations are currently underway.

B. Substrate Utilization and Lactate Clearance Rates

1. Introduction: Previously at relatively low time resolution (1 minute) we have demonstrated that lactate production during 30 consecutive periods of reflow and ischemia follows an approximately exponential increase to a plateau level (section A). To evaluate the determinants of lactate production that give rise to this accumulation profile, we have made rapid serial measurements of lactate (5 or 10 second time resolution) by in vivo \(^1\)H NMR spectroscopy. We have used these measurements to 1) quantitate the ischemic production and clearance rates and 2) use these measured values to model lactate accumulation during thirty sequential periods of ischemia and reflow.

2. Methods: Seven male Sprague Dawley rats (200-240gm) were prepared surgically as described in section II.A.2. A brief (60 second ) sustained occlusion followed by 15 minutes of reflow was used to measure the rate of lactate production during ischemia. The repetitive ischemia protocol consisted of 30 consecutive 30 second (nominal duration) ischemia and 30 second reflow periods followed by 30 minutes of continuous reflow. The one minute occlusion protocol was carried out before the repetitive ischemic protocol.

NMR data was collected using a 4.7T 40cm bore Bruker Biospec system using a single tuned 8x12mm elliptical \(^1\)H surface coil placed directly on the skull. To minimize the acquired lipid signal, the scalp was removed and the temporal muscles retracted. During the sixty second sustained ischemic periods, spectra were acquired with 5 second time resolution (TR = 1250 msec, 4 averages). During the repetitive ischemia protocol and the first ten minutes of continuous reflow, spectra were acquired with 10 second time resolution (TR = 1250 msec, 8
averages). During the final 20 minutes of reflow, spectra were acquired every 30 sec (TR=1250, 24 scans). Resonance areas were determined by peak integration. Resonance areas were converted to millimolar values using a 11 mM value for cortical creatine, and NMR determined values.

Lactate accumulation during the repetitive ischemia period was modeled as the balance between lactate production at a constant rate (\( \frac{d[lactate]}{dt} = k_p \)) and a first order clearance process (\( \frac{d[lactate]}{dt} = -k_c[lactate] \)). The value of \( k_p \) for each animal was determined from a one minute continuous occlusion before the repetitive ischemia protocol. The value of \( k_c \) was determined from the reflow period following thirty cycles of the repetitive ischemia protocol. The exact duration of the ischemic period in each animal was determined from the carotid pressure recordings. Due to the small residual flow experienced during the ischemic period 12 ml·100 g⁻¹·min⁻¹ lactate clearance was assumed to occur continuously throughout the ischemic and reflow intervals. The calculated values for \( k_p \) and \( k_c \) along with the ischemia duration for each animal were then used to predict the time course of lactate accumulation during the repetitive ischemia protocol.

3. Results:

i. Lactate Clearance Kinetics: The rate of lactate clearance was determined in each animal using the equation

\[
[lactate](t) = [lactate]_0 \exp(-k_p t)
\]

where \([lactate]_0\) is the lactate present at the beginning of the thirty minute reflow period and \(t\) is the time in minutes, and \(k_p\) is in units of \(\text{mM min}^{-1}\). The lactate level is reported as a difference from the control period to minimize the effect of resonance overlap with other metabolites and lipids. Displayed in figure 10 is an example of the lactate clearance along with the fitted exponential. The measured clearance rates are reported in table 1 along with the mean and standard deviations (0.143±0.032 mM·min⁻¹) for all seven animals. This value is in good agreement with the lactate transport rate of 0.150 umole·g⁻¹·min⁻¹ reported by Drewes and Gilboe in the perfused dog brain (7).

ii. Lactate Generation Kinetics: The lactate production rate was determined from a one minute continuous occlusion in each animal prior to the repetitive ischemia protocol. Typical 5 second lactate spectra are displayed in figure 11a. The lactate difference data was fit using a linear production rate and a correction for lactate clearance

\[
[lactate](t) = k_p t - k_c[lactate]
\]

where \(k_p\) the production rate in \(\text{mM·min}^{-1}\). The value of \(k_c\) used was determined as previously described. A typical data set is displayed in figure 11b. Calculated values for \(k_p\) for all seven animals are listed in table 1. The values reported here (4.63±0.73m·min⁻¹) are somewhat lower than those reported by Nilsson(8), 7.0 umoles·gm⁻¹·min⁻¹. This may reflect differences in the glycolytic rate arising from anesthesia and or the small residual flow experienced in the repetitive ischemic model (typical CSP = 7-10 mm Hg, figure 12).

iii. Determination of Ischemic Duration: Due to the finite time for inflation and deflation of the occlusive cuff, the actual ischemic periods were found to be shorter than 30 seconds and vary slightly from animal to animal. Additionally, substantial lactate generation or anaerobic glycolysis will only occur after brain oxygen stores have been depleted. Lowry (6) has reported
that lactate generation begins after a 3 second time delay in animals subjected to complete global ischemia. To account for the time delay in inflating the occluder and the subsequent delay to consume brain oxygen stores, the duration of the ischemic period was defined as the time during which the carotid pressure was within 1mm of the ischemic minimum value (figure 12) less three seconds. This correction shortened the mean ischemic duration to 21 ± 1 sec and the individual times for each animal are reported in table 1.

iv. Lactate Accumulation during Repetitive Ischemia: The time course of lactate accumulation during the 30 consecutive ischemia/reflow periods was modeled using the rate constants, \( k_p \) and \( k_c \) and the ischemic duration calculated from the carotid pressure measurements. The incremental change in lactate during each ischemic and reflow period was calculated using the lactate generation rate (\( k_p \)) corrected for the duration of the ischemic period (\( t_{sec}/60 \)) minus clearance during the entire interval (\( k_{lactate} \)).

\[
\text{lactate} = k_p(t_{sec}/60) - k_{lactate} \]

To evaluate the accuracy of the calculated time courses, the observed data, calculated data and calculated data assuming 15% errors in production rate constants are displayed for all seven animals in figure 14. All animals showed excellent agreement, with nearly all measured values lying within the 15% error bounds. Assuming error bounds of 15% in the clearance rate provides similar limits and they are not shown for sake of clarity.

The high sensitivity of the shape and absolute level of lactate accumulation to clearance rate, production rate and ischemic duration and the agreement of the model and observed data suggest that both the model used and the rates measured are highly accurate, (within 15%). The assumption that lactate clearance occurs throughout the “ischemic” period in this model is also supported, since a 33% decrease in reflow time would cause large changes in the total amount of lactate produced.

4. Summary: We have demonstrated that the accumulation of lactate during a brief repetitive ischemia protocol can be accurately modeled (<15% error) using rate constants determined on an individual animal basis. Thus the simple model of lactate production at a constant rate during each ischemic period, and washout throughout the reflow and ischemic period provides an excellent model for predicting the amount of lactate accumulated during the protocol. The observed lactate production during each ischemic period appears to occur at the same rate as during a continuous one minute ischemic episode and these rates are consistent with the maximum glycolytic rates observed by Nilsson (8) in a model of complete global ischemia. Thus, lactate production in this repetitive ischemia model does not appear to be limited by glucose or other substrate availability. This indicates that glucose replenishment during the reflow period is sufficient to support the elevated level of anaerobic activity. This is in contrast to continuous global ischemia models where the amount of lactate produced is dependent on the brain glucose and glycoamines stores immediately prior to the induction of ischemia. Thus glucose availability is unlikely to be a major factor in determining the extent of ischemic damage in this model. However, if lactate accumulations are deleterious, via pH and or osmotic changes, then interventions to alter the efficiency of the monocarboxylic acid transporter may provide a mechanism for tissue preservation. The correlation of lactate accumulation and histologically visualized damage forms the next area of our research.
III. PLANS

A. Regional Measurements of Lactate: As described, our previous studies have focused on the issues of metabolite and functional alterations during the ischemic protocols from the entire brain. However, they have not addressed the issue of focal tissue damage and selective vulnerability. To pursue this question we have implemented a \(^1\)H spectroscopic imaging sequence to evaluate the regional time course of lactate elevation. This method permits us to acquire data from 256 individual spatial locations (approximately one half within the brain) simultaneously during a five minute measurement. The volume elements investigated here are approximately 1x1x4mm. This method will be used to assess the regional time courses of lactate elevation in 20I:40R, 30I:30R and 40I:20R protocols. The lactate time course will be followed throughout the repetitive ischemias and three hours of reflow. A three hour reflow time is chosen here so as to facilitate the histologic evaluation of the damage. One limitation to this method is the large amount of data acquired during such an experiment, typically several thousand brain spectra per animal (100 spectra per time point x 15-20 time points). We are now evaluating the most efficient way of analyzing the large volume of data that are acquired in these studies.

B. Histologic Evaluation of Damage: As described the spectroscopic imaging method will provide a profile of regional lactate elevation. However, this does not address the question of the association of tissue damage and lactate elevation. Therefore we have implemented a quantitative method for evaluating damage. Rat brains are perfusion fixed and sectioned serially using an Oxford Vibratome at 400 microns. Optical densities are recorded using an optical scanner. Increased optical density compared to control levels has been found to correlate with pathological changes. Data from approximately 50 coronal sections are then used to reconstruct absorbance profiles of the brain and the tissue sections are further processed to evaluate neuronal damage in one micron plastic sections (9,10). Data from the histologic evaluations are then overlaid with the lactate maps to assess the degree to which 1) areas of maximum lactate production correlate with damage and 2) the presence of selective vulnerability.
REFERENCES


V. FIGURE LEGENDS

Figure 1.
Block diagram of the components of the occlusion system. The computer monitors the carotid stump pressure (CSP) and systemic blood pressure (SBP) via the left common carotid artery. The ischemia is induced by inflating the cuff placed about the right common carotid artery. Adequate pressure within the cuff is maintained by the pump and regulated through the output of the transducer.

Figure 2.
Systemic blood pressure (SBP) and carotid stump pressure (CSP) as a function of time for a 40l:20R animal. Insets and plots to the right display the time course of 4 ischemic episodes of CSP and SBP for the four ischemic protocols.

Figure 3.
Fast (7-20Hz) and slow (2-5Hz) components of the EEG as a function of time during the ischemic protocol. The values are reported as a fraction of the amplitude prior to induction of ischemia. The response to the first four ischemic periods for the four protocols are shown in the insets and to the right.

Figure 4.
Fast and slow EEG activity averaged over all animals in each protocol. Error bars indicate standard error of the mean (SEM). EEG activity is averaged over the one minute periods of ischemia and reflow and reported as a fraction of the amplitude during the control period.

Figure 5.
$^{31}$P spectra acquired before, at the conclusion of thirty cycles of 40l:20R ischemia and after thirty minutes of reflow. The observed resonances are P, (2-3ppm), PCr -2 ppm, ATP -4.5ppm, -9.5 ppm and -18.5 ppm.

Figure 6.
Average PCr, ATP and P, as a per cent of control level as a function of time during the ischemia. Error bars indicated SEM. P, is plotted as a percent of the control PCr level.

Figure 7.
$^{1}$H spectra acquired before, at the conclusion of thirty cycles of 40l:20R ischemia and after thirty minutes of reflow. The observed resonances are: lactate 1.33 ppm; N-acetyl aspartate 2.02 ppm; glutamate, glutamine, aspartate and N-acetyl aspartate (2.1-2.8 ppm); creatine + phosphocreatine 3.03 ppm and choline 3.18 ppm.

Figure 8.
Lactate and pH as a function of time during the ischemic period. The values represent an average for each ischemic group. The observed pH is plotted as a function of lactate in the third panel.

Figure 9.
Correlations between fast and slow EEG activity as a function of lactate, pH and PCr. The correlation coefficients are listed on each plot.
Lepresenrat iv course of lactate clearance. The observed values (filled circles) for lactate (DELTA LACTATE) are plotted as a function of time after the beginning of ischemia. Thirty minutes corresponds to the beginning of the continuous reflow period. The lactate levels are reported as the difference in areas of the 1.33 ppm resonance from that observed in the control period. The first eight minutes of the clearance data were acquired with 10 second time resolution. The gap in the data from 38 to 41 minutes is the amount of time required for saving the NMR data. Measurements after this point in time were acquired with 30 second time resolution. The fitted exponential clearance curve is indicated by the solid line. The clearance rate $k_c$ was determined using a simplex algorithm and minimizing the squares of the differences of the observed and calculated data.

Figure 11
a) Lactate spectra acquired with five second time resolution, during the control period (first three spectra) and during the first minute of a continuous ischemia. The starting time of the spectrum relative to the beginning of the ischemia is noted on the vertical axis in seconds. The observed resonances are: lactate 1.33 ppm; N-acetyl aspartate 2.02 ppm; glutamate, glutamine, aspartate and N-acetyl aspartate (2.1-2.8 ppm); creatine + phosphocreatine 3.03 ppm and choline 3.18 ppm.

b) Lactate accumulation time course with the observed values (filled circles) for lactate (DELTA LACTATE) as a function of time after ischemia in minutes. The modeled values for lactate accumulation including the clearance term is denoted by the line. The generation rate, $k_p$, was determined using a simplex algorithm and minimizing the squares of the differences between the observed and calculated data.

Figure 12
The CSP from all thirty occlusions from a typical animal were overlaid as a function of their time during the individual ischemic episode. The fall in CSP was modeled as an exponential, whereas the rise in CSP accompanying the release of the occluder was modeled as a linear process. The duration of the occlusion was calculated as the difference between the two fits at a point 1mmHg above the minimum CSP. The minimum CSP was determined from the exponential fit.

Figure 13
The relationship between cerebral blood flow (CBF) and CSP for eight rats. The data is obtained from eight rats implanted with cortical $H_2$ electrodes. The CBF was determined by $H_2$ clearance within 5 to 10 minutes after reduction of the CSP to the specified level. The CSP was maintained at that level by servo controlled compression of the right carotid artery with a balloon occluder.

Figure 14
The observed (solid circles) and calculated lactate data (solid and dashed lines) from the seven experimental animals. The lactate level (Delta Lactate) in mM is plotted as a function of time in minutes after the beginning of the repetitive ischemia protocol. The continuous reflow period was initiated at thirty minutes after beginning the repetitive ischemic protocol. The solid
line indicates the calculated lactate time course using the experimentally determined values of $k_p$ and $k_c$. The calculated time courses assuming a ±15% error in the production rate $k_p$ are indicated by the dashed lines.
TABLE 1

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Mean±std 0.141±0.032 4.63±0.73 21.1±1.0
The diagram shows the activity levels over time in two frequency bands: 2-5 Hz and 7-20 Hz. The data is represented at different time intervals (minutes) with markers indicating specific time points (10:50R, 20:40R, 30:30R, 40:20R). The activity levels are measured in percentage (%).
Fig 9

- $10^2:50^R$
- $20^I:40^R$
- $30^I:30^R$
- $40^I:20^R$

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<td>$r = -0.93$</td>
<td>$r = -0.94$</td>
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<table>
<thead>
<tr>
<th>Lactate (mM)</th>
<th>pH</th>
<th>PCr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
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<td>$r = 0.95$</td>
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OVERLAY OF 30 OCCLUSIONS

Carotid Stump Pressure (mmHg)

Time (seconds)

- EXPONENTIAL FALL OF CSP
- OCC. TIME = 25.66 SEC
- LINEAR RISE
Experimental and Simulated Delta Lactate (mM)

- Data
- Model
- ± 15%

Time (minutes)
Simulating Lactate Accumulation During Repeated Brief Cerebral Ischemia

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Introduction:
Pilots of high performance aircraft are subject to transient loss of consciousness due to sudden high gravitational stress. This is due to cerebral ischemia resulting from failure of blood delivery to the brain due to the severe gravitational effects. We have developed an animal model whereby the problem of multiple Gz-induced blackouts can be investigated. The objective was to determine if the time course of lactate accumulation could be predicted from measurements of glycolytic rate made prior to the multiple occlusion period and measurements of terminal washout rates.

Methods:
A) Experimental: Six rats were anesthetized with 70% N2O, 29% O2, and 1% Halothane. The rats were paralyzed with tubocurarine chloride and mechanically ventilated. Rats were prepared by ligation of both subclavian and external carotid arteries. The right carotid was cannulated distally for monitoring of carotid stump pressure (reflecting cerebral perfusion pressure at the Circle of Willis). Systemic arterial pressure, rectal and mouth temperatures were monitored and mouth temperature was controlled at 37°C. An occlusive cuff was placed around the remaining left carotid (fig. 1). Prior to and following the multi-occlusion experiment rates of lactate generation were determined during one minute test occlusions. Plasma glucose, hematocrit and arterial blood gases were determined immediately prior to the first episode of ischemia. Thirty 30 second episodes of global brain ischemia were made by temporary intubation of the carotid balloon while 1H spectra were acquired at 10 second intervals, 8 acquisitions using a semi-selective spin echo sequence, Te=136us, Te=1.25s, with a 4.7T Bruker Biospec system. Each occlusive period was followed by 30 seconds of reflow.

B) Simulation: The simulation is designed to describe the principal components that contribute to determine the shape of lactate accumulation curves found experimentally in our multi-occlusion model. The simulation is based entirely on experimentally determined values, therefore, no fitting parameters are applied in the simulation. Prior to the multi-occlusion experiment depicted in figure 2 (results), the animal was subjected to a one minute occlusion. A least squares linear fit to the measured change in lactate gave us an estimated 4.58 ± 0.26 mM/min rate for lactate generation during ischemia. To obtain a best estimate of lactate clearance rates, we fit a monoexponential to the lactate washout curve following the multiple occlusions. The rate of lactate loss could be defined as follows:
Lactate loss rate (mM/min) = 0.0875/2 * Lactate(MM)

The experimental results were then simulated for the thirty cycles of occlusion-reperfusion by generating lactate linearly during each occlusion and decreasing lactate exponentially during each reperfusion period.

Results:

The experimental results were then simulated for the thirty cycles of occlusion-reperfusion by generating lactate linearly during each occlusion and decreasing lactate exponentially during each reperfusion period.

Conclusions:
We believe that the occlusive model functions as follows: With each occlusion lactate is generated linearly at the maximum catalytic rate of the glycolytic pathway. Glucose is not exhausted during the short occlusive process, and is subsequently replenished in the short reperfusion periods following occlusion therefore, absolute glucose brain or plasma levels do not affect lactate levels. Lactate concentrations reach plateau levels because lactate clearance increases exponentially until the clearance rate matches lactate generation rates. It has been suggested (1) that the carrier mediated transport of lactate may be the limiting factor responsible for the observed rapid build-up of lactate during hypoxemia and the slow removal of lactate in post-hypoxicemic recovery (2).

Acknowledgments:
This work was supported in part by AFOSR-90-0269 and the Bemhard-Foundation.
Concomitant Lactate, Lactate and Phosphorous Changes During Repeated Brief Cerebral Ischemia

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Abstract

Pilots of high performance aircraft are subject to transient loss of consciousness due to sudden high gravitational stress. This is due to cerebral ischemia resulting from failure of blood delivery to the brain due to the severe gravitational stress. We have investigated the problem of Gz-induced blackout in an animal model in which controlled brief global cerebral ischemia is produced repeatedly at short intervals. The objective was to determine if this results in cumulative impairment of brain metabolism and electrical function. Our initial hypothesis was that accumulation of lactate might be an important element in this process.

Methods:
Animal Model: Rats were anesthetized with 70% NO2, 29% O2, and 1% Halothane, paralyzed and mechanically ventilated. Rats were prepared by ligature of both subclavian and external carotid arteries. The right carotid was cannulated distally for monitoring of carotid stump pressure (reflecting cerebral perfusion pressure at the Circle of Willis). Systemic arterial pressure, rectal and mouth temperatures were monitored and mouth temperature was controlled at 37°C. An occlusive cuff was placed around the remaining left carotid (fig.1). Thirty ischemia:reflow cycles of global brain ischemia were made by temporary inflation of the carotid balloon.

Simultaneous measurements of lactate, high energy phosphates and EEG: We used a two coil design (one 13T Bruker). It was used measurements and a one coil (larger in size but coil) when passing the signal through a high interleaving a experiment. It was different coil (larger in size but coil) than that of the 31P coil just above the 31P coil. The 31P coil is elliptical and placed directly against the skull, the 1H coil is a figure 8 coil (larger in size but due to its geometry, samples approximately the same volume as the 31P coil) just above the 31P coil. The data was acquired in one minute blocks allowing moving 4 minute averages to be used for one 31P data. 31P and 1H data acquisitions are acquired simultaneously throughout the 1 minute block by interleaving a 31P and a 1H scan every half a second with a 4.7T Bruker Biospec system. EEG is acquired continuously throughout this period and quantified by passing the signal through a high pass filter 7 to 20 Hz and digitizing the output every 0.5 seconds (see figure 2, Results).

Results:
A typical pressure and EEG sample from a 30:30 ischemia:reflow experiment is given in figure 2. The results of 20 full experiments are summarized in Figure 3 where a marked quantitative similarity between the patterns of accumulation of lactate and the time course of EEG loss in the 10:50, 20:40 and 30:30 cycle experiments is demonstrated. The shape of the lactate accumulation curve is not mimicked as well in the 40:20 cycle group were an early rapid decline in EEG coincides more closely with early PCR changes (figure 3).

Figure 1. Block diagram of the components in the automatic occluder system. The computer monitors carotid, systemic blood and occluder system pressures. Computer monitoring and control of mouth and rectal temperatures was implemented.

Figure 2. The figure depicts the time course of EEG change and carotid pressure. It is noteworthy that the time course of EEG change and pressure change is not in phase. We set up database extraction techniques which permit automatic calculation of EEG activity during any fraction of the pressure cycle. Figure 3 below illustrates the values obtained by averaging the whole one minute cycle for EEG activity.

Figure 3. The illustration depicts the time course of lactate, PCR and EEG activity using a four minute running average for PCR measurements and a one minute average for lactate and EEG measurements. Plotting symbols represent mean values of five experiments ± SEM for each condition (20 total experiments). Open circles represent means of 10:50 cycles, filled circles of 20:40 cycles, empty squares 30:30 cycles and the filled triangles a 40:20 cycle of ischemia:reflow.

Conclusions:
Both EEG and lactate changes show almost identical kinetics of collapse (accumulation) and recovery (washout). Tentatively one might conclude that lactate levels (or the resulting pH changes) may affect EEG levels in the absence of significant energy loss. However, care must be taken in this interpretation as lactate generation and EEG loss happen at equivalent times and (ie. during the fractional occlusion period) and thus may simply reflect the measurement of a common phenomenon.

Acknowledgement: This work was supported in part by AFOSR-90-0269 and the Bollard Foundation.
Correlating EEG and Lactate Kinetics During Repeated Brief Cerebral Ischemia
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Pilots of high performance aircraft are subject to transient loss of consciousness due to cerebral ischemia resulting from sudden high gravitational stress. To assess the effects of Gz-induced blackout on cerebral metabolism and electrical function we developed an animal model in which global cerebral ischemia is produced repeatedly at short intervals. Rats were prepared by ligation of both subclavian and external carotid arteries and the right carotid artery was cannulated bidirectionally to measure Circle of Willis and systemic pressures. Ischemia was induced by inflation of an occluder about the left carotid artery. Interleaved 31P and 1H were acquired on a 4.7T Biospec system simultaneously with EEG recordings. We report results from 20 experiments of 30 minute duration in which rats were subject to 30 one minute ischemia-reflow cycles of 10:50, 20:40, 30:30 and 40:20 (numbers are seconds of ischemia and reflow respectively). EEG collapse and lactate increase showed nearly identical kinetics. Loss in EEG activity was observed without significant sustained energy loss in all but the most severe cycle. In all animals the lactate was observed to reach a plateau during the ischemic period, the amplitude correlating with the severity of the ischemia, consistent with a model of linear lactate generation and exponential washout. This model was confirmed in seven additional rats by measuring the initial rate of lactate generation and washout in 1 minute test occlusions. These values were then used to predict lactate evolution in 30 minute (30:30) ischemia experiments. In five of the seven animals the experimental time course was simulated adequately (±10%) from experimentally measured lactate generation and clearance rates.

The author affirms that the abstract herein will not have been published as a manuscript prior to presentation at the American Heart Association meeting or presented-or published as an abstract at any national meeting or world congress held in the United States, that any animal studies conform with the “Position of the American Heart Association on Research Animal Use” (Circulation 1985;71:849), and that any human experimentation has been conducted according to a protocol approved by the institutional committee on ethics of human investigation or, if no such committee exists, that it conforms with the principles of the Declaration of Helsinki of the World Medical Association (Clinical Research 1966;14:103).

The submitting author also certifies that all authors named in this abstract have agreed to its submission for presentation at the AHA Scientific Sessions and are familiar with the 10-author rule (see “Rules for Submission of Abstracts”).

1332 Ischemia
1602 Reperfusion

Submitting author’s signature