Strain Dependent Ischemia in Brain Tissue as a Function of Inertial Loading of the Head

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ABSTRACT

Controlled inertial loading of the head was used in an experimental setting producing varying degrees of ischemic changes in the brains of subhuman primates. Sampling from frozen coronal sections of the brain enables one to quantitate the ATP levels of the local specimens and, subsequently, to map the topographical distribution of this biochemical indicator of ischemia.

In parallel with the subhuman primate studies a series of physical model experiments were conducted in order to estimate the magnitude of the strains produced throughout the surrogate brain material under identical loading conditions. Based upon isolated vascular tissue experiments we have been able to demonstrate a strain-dependent vasoconstriction that occurs in the vessel under conditions of high strain rate extension. We, therefore, hypothesized regional changes in cerebral blood flow would accompany changes in the strain field.

We have mapped the strain field onto the topographic distribution of the alterations in ATP levels and have demonstrated a correlation between the magnitude of the principal strains and the degree of ischemic change. This finding should be considered in the context of the development of improved head injury tolerance criteria, in that a threshold exists for ischemic brain damage as a function of acceleration of the head through the mechanism of strain-dependent vasoreactivity.

INTRODUCTION

Cerebral ischemia is a reduction of blood flow below the metabolic demand in the brain, and can lead to failure of the oxygen dependent metabolism in these regions. This condition has been experimentally produced through ligation of a series of blood vessels ascending into the brain. Much of this research [1-7] follows the development of secondary effects in the post ischemic brain from several hours to weeks post insult. There is evidence that links short term, as short as 5 minutes, ischemic insults with decreases in pH and adenosinetriphosphate (ATP) levels[8]. In some ischemic episodes up to 30 minutes, ATP is depleted and only returns to normal after hours of reperfusion[5]. In another study [3], where carotid occlusion was used, ICP levels were raised while maintaining arterial pressure constant. This reduced flow to 70 percent of control but reduced ATP levels to well below 20 percent of control [3]. This occurred without significant impact on other metabolites such as lactate and glucose concentration. The relationship between ischemia and other short and long term changes in tissue metabolites has been explored by many other researchers[9-16]. There seems to be a consistent correlation between a reduction in cerebral perfusion pressure (the difference between ICP and arterial pressure) and ischemic insult severity in animal models. One such study suggests that trauma induces a transient, profound disturbance of energy metabolism in the brain tissue, as a result of mechanically induced disruption of ion homeostasis and reduced blood flow in combination[17]. Studies have linked ischemia after trauma to neural cell death through an excitotoxic mechanism triggered by an intracellular overload of calcium ions[18-21]. In addition, short term ischemia may also directly influence post ischemic blood flow by affecting arterial vascular tone[22].

Gennarelli and Thibault [23, 24] observed increases in intracranial pressure (ICP) immediately following acceleration of the head in experiments that reproduced diffuse axonal injury (DAI) in primates. In addition to ICP, mean blood pressure (MBP) was also recorded. From this data one can calculate the cerebral perfusion pressure (CPP) which is defined as the difference between MBP and ICP. Figure 1 presents the values of CPP for a typical acceleration level of 640 g (peak tangential acceleration). We hypothesize that the change in ICP, and thus the change in CPP, immediately following head injury is related to a transient decrease in intracranial circulation. Since this phenomenon is a nearly immediate response to the mechanical insult, edema and CSF shunting are not likely significant etiologic factors. Rather, we propose that inertial loading of the brain results in stretching of the blood vessels and a venous spasm with concomitant local alterations in cerebral blood flow.

These transient increases in ICP have not been observed clinically due to the short time course of the event; the peak intracranial pressure occurs within ten minutes of the traumatic insult. However, these rises in ICP have been corroborated utilizing the piglet fluid percussion model in which not only increases in ICP were measured but also decreases in cerebral blood flow coupled with increases in cerebral vascular resistance. This study by Pfenninger et al. [25] shows specifically that as ICP increases 4.5 times within 5 minutes of insult, cerebral blood flow decreases by 60 percent in various brain areas in this same time period. In addition, this study also measured an increase in cerebral vascular resistance of 300 percent. Blood flow studies were also performed in the guinea pig optic nerve model of head injury. In these studies, a decrease in blood flow was measured using a laser-doppler blood velocity probe during uniaxial, high strain rate extension of the optic nerve bundle [unpublished work]. In addition, clinical observations of vasospasm [27] have been made in response to mechanical manipulation of vessels during neurosurgery.

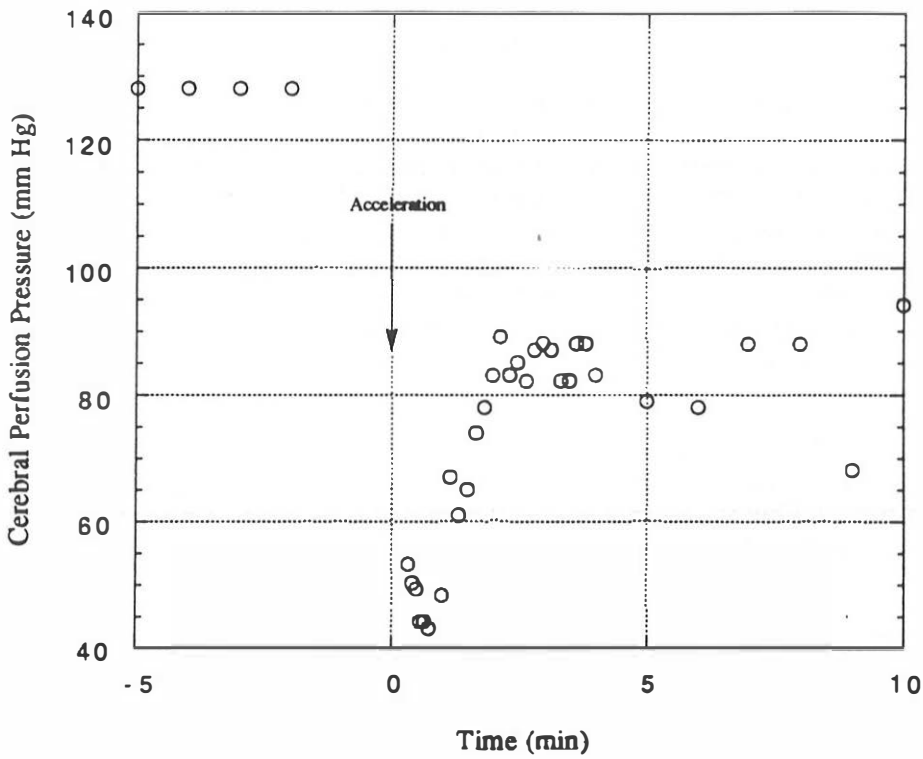


FIGURE 1

In chemical assays conducted on ten primates following inertial loading, various levels of ATP were measured and these relate directly to the level of mechanical loading on the primate and the concomitant distribution of the tissue strains. One explanation is a potential relationship between regional variations in blood flow and the associated compromise to oxidative metabolism operating synergistically with injuries to neural tissue in that region. Elsewhere [28] we have shown that blood vessels respond to high strain loading by producing a spasm in the venous circulation. The range of magnitudes of strain used were comparable to those experienced by the brain macroscopically [29]. We observed a continuum of physiologic response - from the reversible spasm to ultimate structural failure - in response to increasing levels of mechanical strain. The ten primate experiments in which these chemical assays were performed also measured a transient intracranial pressure change. The measured increase in ICP was to levels equal to the mean blood pressure in the animal.

METHODS

Chemical Assays

In previous studies, ten primates (*Papio nubis* n=6 and *Macaca fascularis* n=4) were subjected to a single controlled lateral or sagittal acceleration of the head. The injuries were produced by the Penn I and II HYGE devices [30-32]. The system consists of a 15.2 cm actuator and a kinematic linkage. The device provides a non-impact distributed impulsive

acceleration load to the head through a controlled trajectory using a six inch pneumatic column. This device and procedure have been described in detail elsewhere. The animals were instrumented for monitoring standard physiological variables under intraperitoneal phencyclidine or ketamine and endotracheal nitrous oxide anesthesia. All of these animals were sacrificed two hours post injury in order to do metabolite chemistry. Prior to sacrifice the animal's skull was exposed to allow access for the funnel freeze procedure. After sacrifice the animal was decapitated to stop blood perfusion at a known time. The brain was then removed and frozen using liquid freon. The samples were kept at -85 °C until processing. The brain was then cut in a cryostat maintained at -30 °C. The brain was sliced into 20μ sections within 6-10 hours of the experiment. The brain sections then had 2-5 gram samples taken at various locations using a small cork borer. In addition, several non-injured control animals were sacrificed, sectioned, and sampled using the same technique. All of the slices were then photographed with the location of samples evident. These photographs were hand-digitized using a Macintosh IIci computer and a Summagraphics digitizing tablet. These figures are shown in the results section. These samples were then acid-extracted and the extracts analyzed for various tissue metabolites using enzymatic methods. These values were compared to the control values to show a percentile value of the control metabolite.

Physical Model Simulations

An open ended aluminium cylindrical shell is machined to fit into the linkage of the HYGE machine. A baboon skull was sliced coronally into parts and half of the skull was secured in place within the aluminium shell with a potting resin. An optically clear gel material is then poured into the skulls. This gel serves as a surrogate brain material with a reasonable match in its material properties to brain tissue. The gel also has certain properties of self adhesion that allow pouring to be done in layers. In a layer equivalent to the middle of the skull, the gel has a grid network painted on its surface. The gel filled skull is then subjected to impulsive loads similar to those used in the animal experiments. The grid within the skull is photographed with a high speed motion picture camera (approximately 6600 frames per second). Individual frames of the film are developed into still pictures which then have the grid elements hand digitized into a computer. Any grid element can then be tracked over time and the shear strains calculated across the grid element. The locations of the ATP sampling were then correlated to physical model experiments delineating the strain field of the brain undergoing similar accelerations to the animal experiments [29]. The shear strains were converted to principal strain values. These principal strain values are the basis for correlation to experiments on viable blood vessels reported elsewhere.

RESULTS

Cerebral Perfusion Pressure

Cerebral perfusion pressure is the difference between mean blood pressure and mean ICP. This pressure difference is a measure of the driving force for blood flow in the brain. For purposes of this study we defined an ICP ratio which is the ratio of the peak ICP measured post injury over the baseline ICP measured before injury. We have then correlated the peak tangential acceleration of the loading condition with this ratio of maximum ICP to baseline ICP. Figure 2 shows a linear correlation between external inertial load and the ICP ratio.

Digitized Brain Slices

Following the procedure outlined previously the animal brains were frozen and sectioned. The section then had punch-bob core samples of approximately 2-5 grams taken at various locations of interest. Before removal of the samples, the entire section was photographed with the location of sample sites clearly marked. The samples were then acid

extracted and enzymatically analyzed for ATP, PCr, and lactate. These photographs were then digitized. We chose to concentrate on the analysis of ATP in this report due to the precision and overall reliability of this measurement. We present the data on these figures as percentages of control values.

Figure 3 shows two representative slices from animal B-36. This animal was subjected to a peak tangential acceleration of 1280 g. The lowest levels of ATP in the first section, 15 and 20 percent of control, are found in the region of the corpus callosum. Also in the first section, significant ATP reductions are noted at the surface on the right side of the section. In the second section, values of 15 and 6 percent of control are also found in the region of the corpus callosum. The entire second section has reductions to less than 50 percent of control.

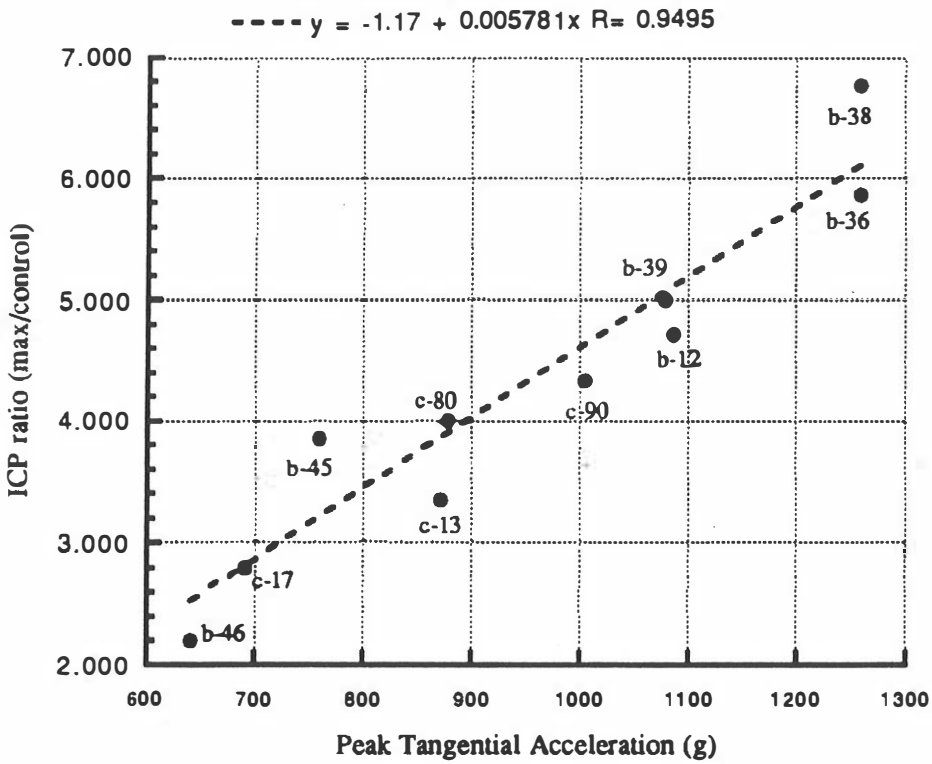
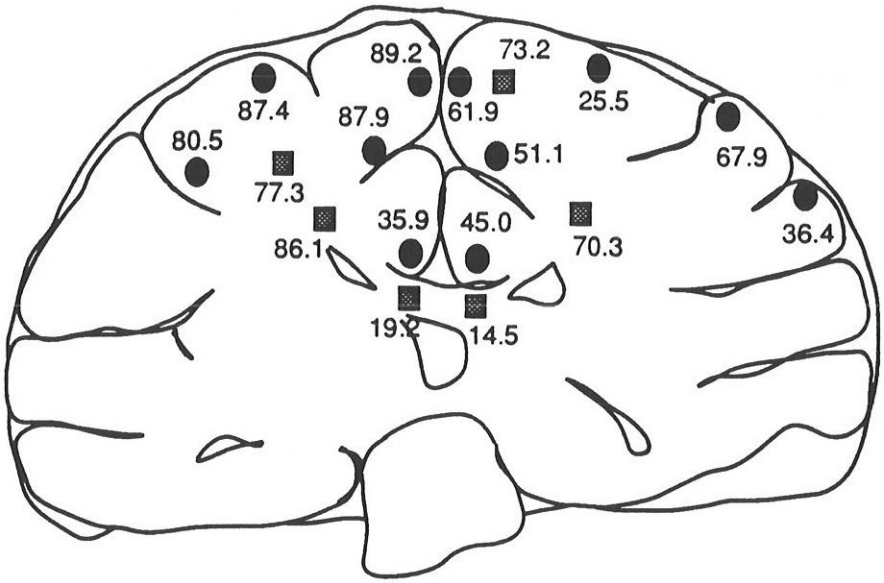
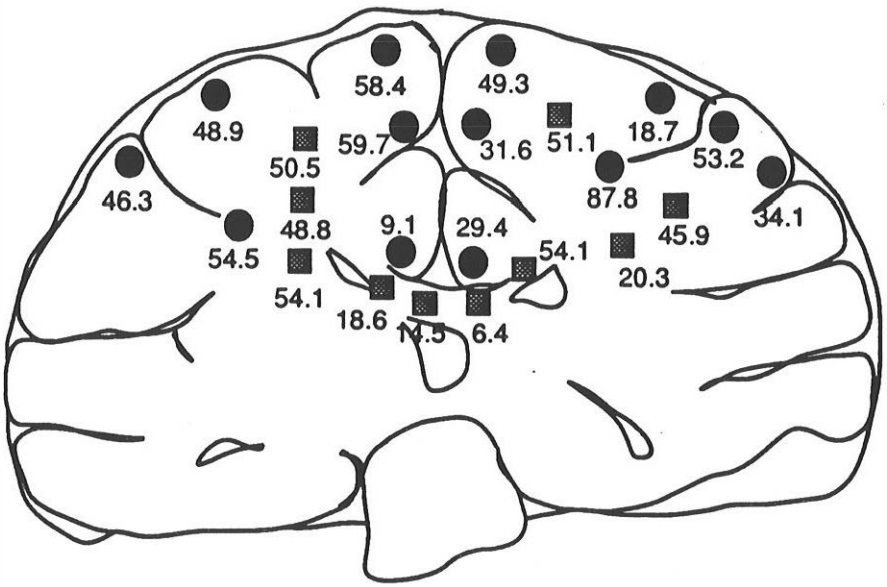


FIGURE 2



These numbers represent % of ATP present
in specimen as compared with control values.



These numbers represent % of ATP present
in specimen as compared with control values.

FIGURE 3

Grid Overlays and Physical Model Correlations

The values of ATP were compared with peak values of the principal strain at locations within the grid of the physical model simulations. The physical model simulations (model B2) were done previously in this laboratory by Margulies[29]. The detailed methods and materials of these experiments along with other details of this work has been described elsewhere. In her simulations, Margulies correlated shear strains to mappings of diffuse axonal injury locations. The shear strains calculated were reduced to principal strains by the eigenvalue calculation. The relationship between these quantities is given as follows:

$$(\varepsilon_{ij} - \lambda\, \delta_{ij})\, v_j = 0$$

or

$$\mathrm{Det}\begin{bmatrix} \varepsilon_{11} - \lambda & \varepsilon_{12} \\ \varepsilon_{21} & \varepsilon_{22} - \lambda \end{bmatrix} = 0$$

where

ε are the calculated shear strains,

λ are the eigenvalues or the principal strains, and

v are the eigenvectors or principal directions.

A representative figure of a time history of a single grid element is shown as Figure 4. The principal strains are used to more closely correspond to the in vitro conditions of the blood vessel experiments and remove the uncertainties with directionality involved in shear strain calculations. The digitized brain slices have had the same grid from the physical model (B2) overlayed upon it. Figure 5 details the overlay grid which is superimposed for reference upon the digitized brain slices. This allows for precise location within the grid elements and subsequent strain calculations for these specific sites of interest. The region of interest in this study consists of a grid area 4 by 5 in the lower mid-central portion of all the slices. This allows several ATP measurements versus several strains at specific grid elements for a single animal to be compared.

The correlation between ATP levels and maximum principal strain are given in Figure 6. This shows good agreement with a power law curve fit through the data. A natural separation of the data seems to occur at 12 percent strain and 15 percent strain. The data below 12 percent represents mild ischemic injury and would compare with the in vitro data representing low (<10 percent) and medium- low strain (10-12 percent). The data between 12 and 15 percent are characteristic of moderate ischemic injury and represent the in vitro data of medium-high strain (13-18 percent). The data above 15 percent represent severe ischemic injury and is representative of the high strain data (>18 percent). We also present the correlation between maximum principal strain and peak tangential acceleration as Figure 7.

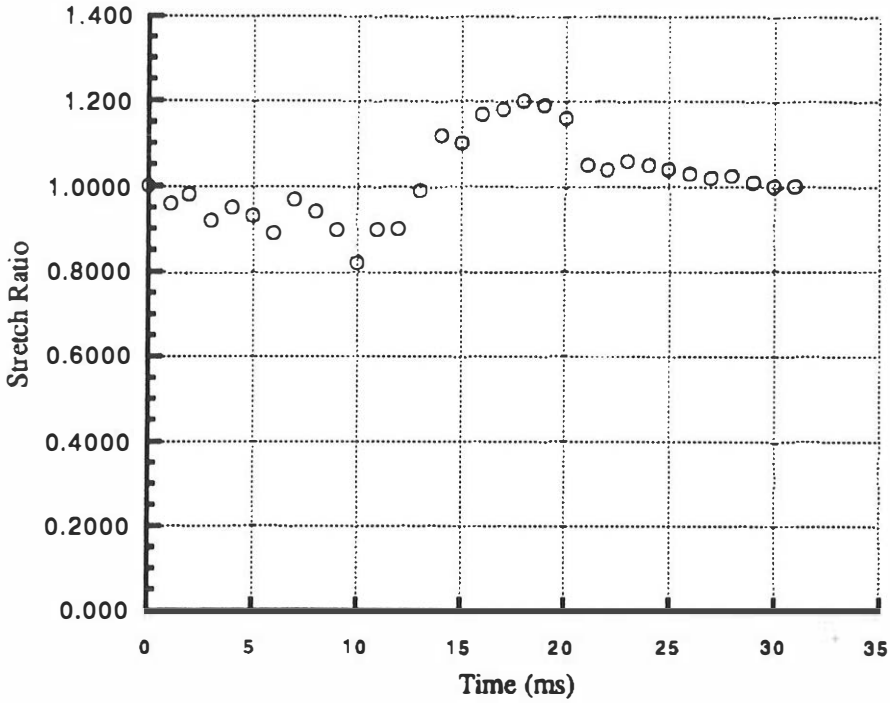


FIGURE 4

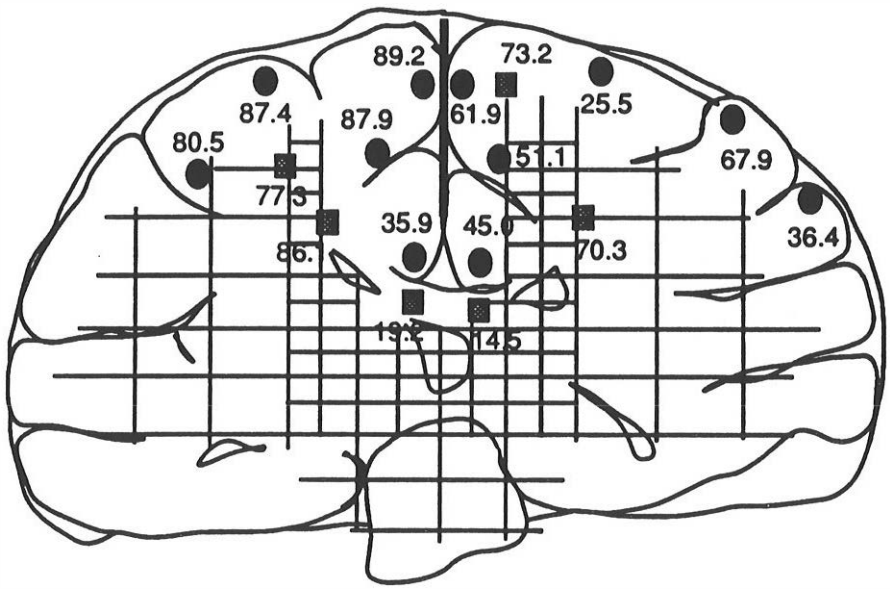


FIGURE 5

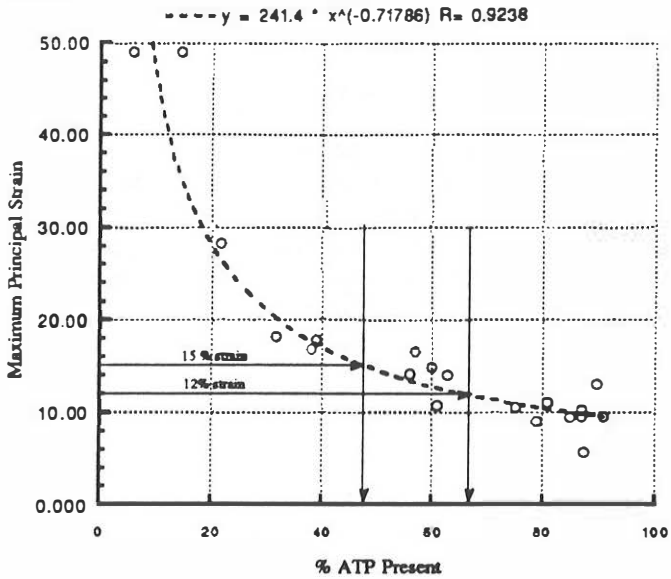


FIGURE 6

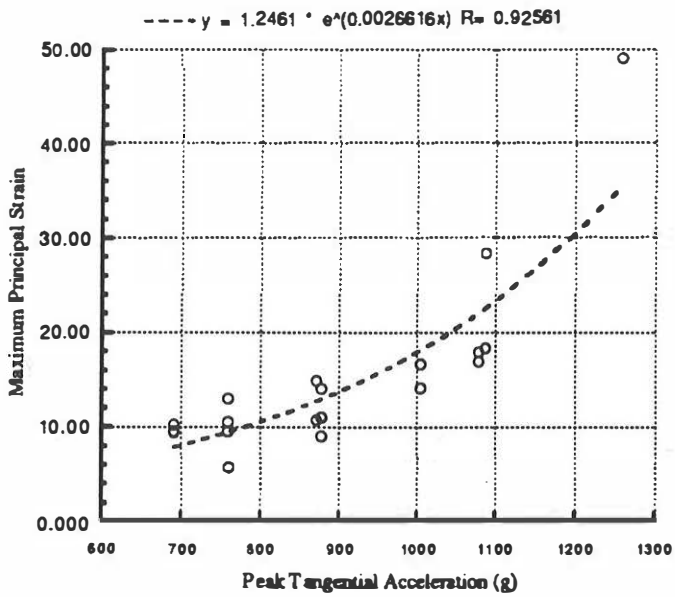


FIGURE 7

The relation between ATP and blood flow changes is unclear in these experimental comparisons because of unknown ATP requirements for injured neural cells. There is some evidence that only a portion of increased ATP usage goes to restoration of ion homeostasis and that this may possibly be explained by changes in intracellular uses of ATP. There is a link between neural cell unexcitability and change in ATP use. The extra ATP required may actually come in part from intracellular stores diverted from cellular electrical activity [1, 5]. Therefore, only a portion of the ATP decrease in the core samples seen must be used to help neural cell recovery. However, another portion of ATP depletion can be readily correlated to reduced blood flow in these areas. Other studies have shown that even small changes in cerebral blood flow can readily deplete all ATP in neural tissue. The long term and secondary consequences of these depletions of ATP and neural recovery cannot be overlooked. The mechanical disruption of blood flow may be the initiator of the cascade of secondary effects already shown to be linked to long term vasospasm, edema, and neural cell death.

CONCLUSION

The physical model strain data from simulation of animal experiments were correlated to show a relationship between regional principal strains and metabolic changes. In addition, the strain values of the physical model simulations can be related to experiments on in vitro blood vessel models which show a vasoconstriction response to uniaxial strains. This vasoconstriction produces regional blood flow changes via alterations in the diameter and hence the effective area for flow of the blood vessels. At the same time these alterations in blood flow occur, other neural tissues are injured and exhibit increased metabolic demands. The reduced blood flow in these tissues has a negative effect on the metabolically driven attempts at recovery. This suggest a synergistic effect between a vascular response to injury and neural tissue responses to injury.

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