LOCAL CHANGES IN BRAIN pH FOLLOWING ISCHEMIA/Kobaiake et al

CEREBRAL VASCULAR DISEASE is the third leading cause of death in the United States and cerebral integrity is at risk in a variety of clinical settings, including: spontaneous cerebral vascular events, resuscitation from cardiac arrest and during certain common surgical procedures such as operations on the cerebral vasculature and open heart surgery. While statistically uncommon, the risk during these operations is, in aggregate, quite substantial due to the great number of such procedures performed annually.

Although the problem of cerebral protection in these and other clinical situations has not been widely accepted, a great number of therapeutic procedures have been employed in an empiric fashion but have inconsistent efficacy and modest success, however despite occasional encouraging reports of cerebral protection these efforts as well have not met with overwhelming success or widespread acceptance. To date virtually all forms of cerebral protection used clinically involve such procedures as: anticoagulation or antiplatelet therapy, vasodilators, mechanical shunts, volume expansion/hemodilution and administration of barbiturates and/or cooling to decrease cerebral metabolism. Other miscellaneous therapies including steroids, osmotic agents or hyperbaric oxygen are variably utilized in empiric fashion but have inconsistent efficacy and have not been widely accepted.

1,3-butanediol, an ethanol dimer, represents a fundamentally different approach to the problem of cerebral protection; it produces a shift in the essential substance the brain metabolizes. Administered intravenously to animals in the pre-ischemic state, the agent produces a marked increase in cerebral blood flow, oxygen and glucose availability, and the absence of delayed post-ischemic neurological deficit.

This study was supported in part by the Michigan Heart Association and the National Institutes of Health Grants HL-16330 and HL-07073.

SUMMARY To determine if 1,3-butanediol (BD), which protects mice from hypoxia, would extend the tolerance of rats to ischemic-hypoxia, the Levine rat (unilateral carotid ligation and conscious hypoxic exposure) was modified to record mean arterial pressure (BP), heart rate (HR), central venous pressure (CVP), spontaneous respiration and EEC. Age and weight matched, male, Sprague-Dawley rats were anesthetized under halothane (1-2%), ligated, instrumented, and recovered 2 hrs before hypoxia (4.5% oxygen). Thirty minutes prior to hypoxia, groups of rats received, BD (47 mmoles/kg i.v.; n = 7), equal volumes of saline (S) (n = 6) or no-infusion (NI) (n = 7). Since no significant difference was observed between S and NI they were combined into a single control group (C). In a parallel group administered BD, resultant beta-hydroxybutyrate (BHB) levels increased from 0.13 ± 0.02 to 0.84 ± 0.03 mEq/L and temperature declined only 1.5°C. The EEG of all ischemic-hypoxic rats invariably became isoelectric before cessation of spontaneous respiration and eventual loss of BP. BD significantly (p < 0.01, Student's t) increased ischemic-hypoxic tolerance (time to isoelectric EEG) from 875 ± 56 seconds for the control group to 1338 ± 67 seconds for the BD group, without changing the interval from isoelectric EEG to loss of BP. Further, EEG activity persisted at a lower mean BP (p < 0.01) in the BD group (44 ± 5 mm Hg) than in the control group (66 ± 4 mm Hg). In summary, isoelectric EEG invariably precedes ventilatory failure and cardiovascular collapse. BD increases ischemic-hypoxic tolerance in the conscious rat by extending, at a lower mean BP, the time to isoelectric EEG.

Butanediol Induced Cerebral Protection from Ischemic-Hypoxia in the Instrumented Levine Rat

E.F. LUNDY, M.D., B.A. LUYCKX, D.J. COMBS, G.B. ZELENOCK, M.D., AND L.G. D'ALECY, D.M.D., PH.D.

References

intravenously butanediol is converted by the enzyme, alcohol dehydrogenase, to the endogenous ketone beta-hydroxybutyrate,29 the latter exists in equilibrium with acetocetate. Ketones are readily utilized as an alternate substrate for brain metabolism.30-32

Previous studies from this laboratory33-34 have shown that blood ketone levels are elevated following butanediol administration and hypoxic survival time in mice is prolonged.33-35 These studies in mice did not delineate important cardiovascular, respiratory and cerebral interactions.

For this study we evaluated the effect of 1,3-butanediol on our modification of the Levine rat preparation which monitors the major physiological parameters of heart rate, blood pressure, respiratory rate, and spontaneous electrical activity (EEG) in the conscious animal.

Materials and Methods

Adult, male Sprague-Dawley rats, carefully controlled for age (67 ± 4.5 days) and weight (314 ± 28 g) were used. Halothane (2%) anesthesia (chamber induction, mask maintenance) was used for the surgical preparation. Right carotid arterial and right jugular venous cannulations were accomplished using saline-flushed PE 50 tubing. The catheters were inserted 2–2.5 cm, secured to the cervical musculature and passed through a subcutaneous tunnel to the nape of the neck. Electroencephalographic leads were placed after first shaving and incising the skin over the dorsal skull. The overlying connective tissue was then cleared and two small burr holes were drilled 2 mm caudal to the anterior suture and 2 mm lateral to the sagittal suture while a third was drilled 2 mm cephalad to the posterior suture and 2 mm lateral to the sagittal suture on the right dorsal skull. Self-tapping 1/8 (4.76 mm) inch stainless steel screws, silver soldered to 30 ga. multi-stranded stainless steel wire were inserted into the burr holes. These EEG screws were isolated from surrounding tissues with dental acrylic. The wires were then passed via a subcutaneous tunnel to exit at the nape of the neck adjacent to the vascular cannulae. The incisions were closed with wound clips and a two hour recovery period was allowed.

Experimental Environment

The experimental environment consisted of a cylindrical clear plastic chamber measuring 30 cm in length and 10 cm in diameter with large rubber stoppers at each end. The chamber was connected via polyethylene tubing to 20.9% O2 (room air) or 4.5% O2 (experimental conditions) premixed gas and had two exhaust ports in the posterior stopper through which EEG leads, vascular cannulae and the rat tail were passed. The latter was secured with tape. Continuous on-line monitoring of the oxygen tension of the gas flushing the experimental chamber was performed using a Beckman OM-14 oxygen analyzer.

The outputs from the vascular cannulae and EEG leads were connected to appropriate transducers and fed into a multichannel recorder (Grass Model 7D Polygraph) in order to continuously record blood pressure (BP), heart rate (HR), respiratory rate (RR), central venous pressure (CVP), and EEG. The cannulae were likewise available for blood sampling.

Protocol

Three groups of rats were tested. No-infusion animals (n = 7) received no pretreatment prior to hypoxic exposure. BD treated animals (n = 7) received 1,3-butanediol (47 mmoles/kg, i.v.) 30 minutes prior to hypoxic exposure and saline treated animals (n = 6) received an equivalent volume of saline as the BD group 30 minutes prior to hypoxic exposure.

The degree of ketosis after BD infusion was determined utilizing three BD treated rats and four saline injected rats. Beta-hydroxybutyrate (BHB) levels were measured after exposure to hypoxia. Immediately after the rats expired, 0.7 ml of blood was removed from the jugular catheter, deproteinized, neutralized, centrifuged, and analyzed for BHB.

In another distinct study aiming to determine the time-course of ketosis induced by BD and its effect on body temperature, three rats were anesthetized with 2% Halothane, underwent right jugular cannulation, were monitored for temperature via a rectal thermistor, and received BD (47 mmoles/kg) after a 30 minute recovery period. Blood samples (0.7 ml), withdrawn from the CVP catheter at: 0, 5, 15, 20, 30, 60, and 120 minutes post-injection were analyzed for BHB exactly as above and temperature was recorded at those same time points with a YSI telethermometer.

Data Analysis

Statistical analysis was performed with the aid of the Michigan Interactive Data Analysis System (MIDAS) on an Amdahl 470/V6 computer. Comparison of each group's overall survival time and the subintervals from onset of hypoxia to iso-EEG and from iso-EEG to loss of cardiac activity (no detectable BP or HR) were done with a Student's t-test. All values are expressed as mean ± one standard error of the mean (SEM); sample size is designated (n).

Results

Following hypoxic exposure, a distinct pattern occurred regardless of the treatment protocol (fig. 1). Loss of cortical electrical activity (iso-EEG) invariably preceded loss of spontaneous respiratory activity and finally, an abrupt loss of cardiac activity with a precipitous loss of BP. As there were no statistical differences in BP, iso-EEG and RR between the no-infusion and saline groups, they were combined and considered as a single control group (C) for subsequent analysis.

Pretreatment with 1,3-butanediol significantly (p < 0.01) increased hypoxic survival time. This increase was manifested as an increase in the interval from the onset of the hypoxic insult to the occurrence of iso-EEG (BD = 1338 ± 67 sec, C = 875 ± 56 sec). There was no concomitant significant difference in the interval from iso-EEG to the last breath (fig. 1) between C (84 ± 21.0 sec) and BD (62 ± 11.7 sec).
nor was there a significant difference observed in the interval from last respiration to “zero BP” (fig. 1) between C (71 ± 6.5 sec) and BD (54 ± 18.6 sec).

Equally important, BD treated groups were able to maintain cerebral electrical activity at a significantly (p < 0.01) lower mean BP (44 ± 5 mmHg) than control groups (66 ± 3.9 mmHg) (fig. 2).

Although mean BP before hypoxia and five minutes after the onset of hypoxia was statistically lower (p < 0.01) in the BD group than in the C group (probably due to the general vasodilatory effect of the alcohol), this difference was no longer observed ten minutes after onset of hypoxia (fig. 2). No significant difference in the pre-hypoxia HR was observed between either of the three groups (no-infusion, saline-injection, and BD-injection). With the onset of hypoxia, however, the no-infusion group exhibited a significantly (p < 0.01) higher HR than both the saline and BD treated groups at 5 and 10 minutes after onset of hypoxia (fig. 3). This difference was only observed at these two time points and, by 15 minutes, was no longer present. The RR was significantly lower (p < 0.01) for the BD group (80 ± 5.2 breaths/min) as opposed to the control group (106 ± 6.0 breaths/min) before onset of hypoxia but this difference disappeared by 5 minutes after onset of hypoxia (fig. 4).

In four saline control and three BD treated rats BHB levels were determined immediately after death. The BHB levels of the BD and saline groups were 0.83 ± 0.04, 0.3 ± 0.03, respectively. These were significant to a level of p < 0.01 (fig. 5).

The time course of the increase in blood BHB levels in response to BD infusion is demonstrated in figure 6. Following i.v. administration of BD, BHB levels rise rapidly and remain elevated for the duration of the experiment. After BD administration rectal temperature did not change more than 1.5°C over the two hour period.

Discussion

In previous studies using the hypoxic mouse model, BD administration increased hypoxic survival time by as much as 560%. Despite economy and reproducibility the mouse model has definite limitations. The small size makes it a technically difficult preparation to instrument for physiologic monitoring; critical cerebral-cardiac-respiratory interactions remain ill defined; there is only one end-point, death; and the model is one of pure hypoxia, an infrequent clinical problem. The more relevant clinical problem is not protection from hypoxia but rather ischemic-hypoxia. We therefore tested BD in a modified Levine rat to better approximate an acute ischemic-hypoxic event.

The Levine preparation (carotid ligation, hypoxic exposure) is a frequently cited model of ischemic-hypoxia which allows comparative assays and is particularly suitable for biochemical and pathological studies. However, as originally described the Levine preparation did not allow for the monitoring of important cardiovascular, respiratory and cerebral interactions. We modified the preparation to enable the monitoring of such parameters; the requisite cannulations also allow access to the central arterial and venous systems. In contrast to our earlier mouse studies the modified rat model has the added component of ischemia; has showed that BD is protective in a second species and has allowed us to monitor the sequence of physiologic collapse. Perhaps most important to the question

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** This bar graph represents the time intervals (sec) to isoelectric EEG, last respiration and "0 blood pressure" for control (white) and 1,3 butanediol (hatched) treated groups. The graph illustrates that pretreatment with 1,3 butanediol (BD) significantly increases the overall ischemic-hypoxic survival time by increasing the interval to isoelectric EEG. In this and all subsequent figures the lines above the bars depict one standard error of the mean. The stars indicate p < 0.01 in comparing control with BD groups by the Student’s t-test and the sample size (n) is in parentheses.

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** This graph plots mean arterial pressure (mmHg) vs. duration of hypoxia (min). The control group is represented by a solid line and the 1,3 butanediol treated group by an interrupted line. The two vertical-hatched areas represent the mean time at which iso-EEG occurred ± one standard error of the mean for the control and BD groups as indicated. The graph reveals that BD treated rats were able to maintain cerebral electrical activity at a significantly (p < 0.01) lower mean blood pressure than control rats. Sample size (n) is indicated by parentheses. One star indicates p < 0.01 in comparing control with BD groups by the Student’s t-test.
of clinical relevance is the fact that this preparation is tested in the conscious, unanesthetized animal. In these initial experiments using this modified preparation we chose to focus on survival rather than production of a lateralized deficit as a necessary first step in establishing the efficacy of BD in the rat ischemic-hypoxia model as opposed to the hypoxic-hypoxia of the mouse model.

In these experiments, all rats subjected to the ischemic-hypoxic insult, demonstrated a consistent sequence of events. First, the EEG became isoelectric, followed by loss of spontaneous respiration, followed by ultimate loss of cardiac activity. This stereotyped response is similar to that reported in canine euthanasia studies using nitrogen inhalation in which an identical sequence was observed.

The initial cardiovascular compensatory activity at onset of hypoxia is a transient rise or no change in BP. With the continuation of hypoxia there is a gradual fall in mean BP due to vasodilation and preterminally a precipitous drop in BP due to cardiac decompensation. With a fully dilated cerebrovascular bed the rate of cerebral blood flow is markedly influenced by the cerebral perfusion pressure. As blood pressure falls in this pressure-passive system, cerebral blood flow decreases to a point at which the inadequate perfusion/oxygenation no longer sustains the brain’s spontaneous electrical activity. Astrup and Siesjo have demonstrated that the flow threshold between loss of electrical activity (0.17 ml/g/min), a reversible deficit, and the irreversible loss of ion pumping/metabolic ac-

**Figure 3.** This graph plots heart rate (beats/min) vs. duration of hypoxia (min). The control group is divided into the no infusion and saline infusion groups to illustrate any possible volume effect. Sample size (n) is indicated by parentheses. One star indicates p < 0.01 in comparing no infusion with saline and BD groups by the Student’s t-test.

**Figure 4.** This graph plots respiratory rate (breaths/min) vs. duration of hypoxia (min). The control group is represented by a solid line and BD treated group by an interrupted line. The two hatched areas represent the time at which iso-EEG occurred ± one standard error of the mean for the control and BD groups. The graph illustrates that loss of cortical electrical activity (iso-EEG) precedes loss of spontaneous respiratory activity. Sample size (n) is indicated by parentheses. One star indicates p < 0.01 in comparing control with BD treated groups by Student’s t-test.

**Figure 5.** This bar graph represents the blood D-beta-hydroxybutyrate (D-BHB) level (mM) for saline and BD treated rats determined immediately after death from exposure to hypoxia. One star indicates p < 0.01 in comparing saline with BD treated groups by Student’s t-test.

**Figure 6.** This graph plots the blood D-beta-hydroxybutyrate (D-BHB) level (mM) vs time (min) from injection with 1,3 butanediol. The graph illustrates that after injection of BD blood D-BHB levels rise rapidly (5 min) and remain significantly elevated longer than any of the hypoxic trials. The sample size is 3 at each time point.
BD OFFERS CEREBRAL PROTECTION/Lundy et al

activity (0.10 ml/g/min) is narrow. In this study direct correlations with cerebral blood flow were not made and loss of recorded electrical activity is being interpreted as cessation of global cerebral function. Inasmuch as respiration failed at a consistent interval subsequent to iso-EEG, we are led to conclude that loss of recorded electrical activity heralds the loss of medullary respiratory center function. The sequence indicates that the ischemic-hypoxic insult impairs brain function, as indicated by iso-EEG, which then precipitates respiratory collapse. We have as yet no data on the structural integrity of the brain under these conditions.

At a constant interval following loss of respiration, blood pressure drops precipitously, presumably because cessation of respiration produces a profound aggravation of hypoxia (less than 20 mmHg) which precipitates cardiovascular collapse due to the combination of ventricular failure and the loss of medullary center activity. Previous studies in rats reveal that an inspired O₂ concentration of 6% reduces arterial Po₂ to 21 mmHg in respiring animals. In this study, rats exposed to 4.5% O₂, with loss of respiratory function, must have an arterial Po₂ that falls below 21 mmHg. Downing has shown that with an arterial Po₂ reduced to 25-35 mmHg, left ventricular contractility is significantly impaired.

Mean blood pressure at prehypoxia and 5 min after onset of hypoxia was significantly lower for BD treated groups as opposed to control. This is most likely explained by an alcohol-like generalized vasodilation and not a specific cardiac depression.

BD rats maintained spontaneous cerebral electrical activity at a significantly lower BP than the control group. At a Po₂ less than 21 mmHg, presumably the cerebrovascular bed was maximally dilated and the autoregulatory mechanism inactive. Cerebral blood flow would therefore be primarily determined by mean arterial pressure. That the BD group was able to maintain spontaneous electrical activity at a lower mean BP (presumably a lower cerebral flow) and that the prolonged survival in the BD group was accomplished solely by increasing the interval from onset of hypoxia to iso-EEG whereas the interval to loss of spontaneous respiration and cardiovascular collapse were constant, strongly implies a specific cerebral protective effect.

In this model, the brain is the critical organ and it is the ability of BD to provide a cerebral protective effect which increases tolerance to ischemic hypoxia. Whether BD affords a measure of cardiac protection or if myocardial function is prolonged solely due to sustained brain and respiratory function cannot be resolved from this experiment. We cannot exclude the possibility of a cardiac protective effect and no definitive statement can be made regarding absolute brain substrate delivery without measurement of cerebral blood flow.

BD is converted to beta-hydroxybutyrate by the enzyme alcohol dehydrogenase. In this model significant increases in BHB levels occurred within five minutes of i.v. BD administration and remained elevated for the duration of the experiment. BHB is one of the endogenous ketones that can be converted to acetyl-CoA in numerous tissues including brain, heart and liver. While this intact animal study does not specifically address the cellular mechanism of increased survival in BD treated rats, other work in our laboratory has revealed that under conditions of relative hypoxia rat brain tissue slices are stimulated to utilize ketones in preference to glucose in the production of carbon dioxide thus supporting the idea that this supplemental brain substrate is involved in the increased tolerance.

BD pretreatment and measurement of relatively objective and discrete end points (isoelectric EEG and loss of respiratory and cardiac activity), were utilized in this study and strongly suggested that BD did provide cerebral protection. In future studies the efficacy of BD as a cerebral protective agent will be assessed when administered subsequent to the onset of an ischemic-hypoxic challenge, the maximal post-ischemic interval at which BD could be effective will be determined and the endpoints will also include the somewhat subjective but more relevant, fixed lateralized neurologic deficits in rats recovered after sublethal ischemic-hypoxic insults.

If BD proves as effective when used in the treatment mode as it has prophylactically, it will have great implications for interventions in such clinically common acute ischemic events as stroke, stroke in evolution, and transient ischemic attacks and perhaps as a protective agent during the temporary vulnerable periods associated with carotid clamping and or cardiopulmonary bypass.

In summary the purpose of this experiment was to determine if pretreatment with butanediol would prolong the survival time in an ischemic-hypoxic model, modified to include line monitoring of BP, HR, RR, and EEG, to define important cardiovascular, respiratory and cerebral interactions. We found that 1) loss of cerebral electrical activity (iso-EEG) invariably precedes loss of spontaneous respiration and ultimate loss of cardiac activity; 2) that pretreatment with BD prolongs overall survival time by increasing the interval from onset of hypoxia to iso-EEG; 3) that cerebral electrical activity is maintained at a lower mean BP following BD treatment and, 4) intravenous BD causes a significant increase in blood ketone (BHB) levels within minutes after administration. Our data strongly supports a significant cerebral protective effect for 1,3-butanediol.

Acknowledgements

We would like to thank Dennis Martin, Co-director of the Biochemistry Core Laboratory in the Michigan Diabetes Research Training Center, for his determination of blood chemistries and Anita Burke and Kathy Simpson for assistance with illustrations.

References

Butanediol induced cerebral protection from ischemic-hypoxia in the instrumented Levine rat.
E F Lundy, B A Luyckx, D J Combs, G B Zelenock and L G D’Alecy

Stroke. 1984;15:547-552
doi: 10.1161/01.STR.15.3.547

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/15/3/547

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/