Ischemic Brain Damage Is Not Ameliorated by 1,3-Butanediol in Hyperglycemic Rats

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Background and Purpose: **Treatment with the ketone body precursor 1,3-butanediol has been suggested to ameliorate hypoxic/ischemic brain damage. Butanediol could provide an alternative energy substrate for the brain, thereby decreasing the amount of glycolytically produced lactate. Hyperglycemia aggravates brain damage after brain ischemia and causes fatal postischemic seizures, probably by increasing the production of lactate and decreasing the pH. We studied whether butanediol treatment altered the adverse consequences following ischemia complicated by hyperglycemia.**

Methods: **Hyperglycemic adult male rats were given 25 or 50 mmol · kg^{-1} body wt butanediol intravenously 30 minutes before 10 minutes of transient forebrain ischemia. Morphological evaluation was performed following perfusion-fixation after 15 hours of recovery. Blood concentrations of β-hydroxybutyrate, acetoacetate, glucose, and lactate and brain tissue concentrations of energy metabolites were measured before and after ischemia.**

Results: **Blood levels of ketone bodies increased in the butanediol-treated rats. Ischemia decreased the blood levels of acetoacetate but increased the levels of β-hydroxybutyrate by a similar amount, resulting in unchanged high levels of total ketone bodies in the animals that received butanediol. Brain tissue levels of glucose, energy metabolites, and lactate showed no difference between butanediol- and saline-treated rats. Furthermore, compared with saline-treated animals butanediol-treated rats showed no decrease in brain damage and no attenuation in the development of postischemic seizures.**

Conclusions: **The ketone body precursor 1,3-butanediol offers no protective effect in transient forebrain ischemia complicated by hyperglycemia. (*Stroke* 1992;23:719–724)**

KEY WORDS • **butanediols** • **cerebral ischemia** • **ketones** • **neuronal damage** • **rats**

There is now considerable evidence that preischemic hyperglycemia aggravates ischemic brain damage in adult animals.[1–4] Cardinal features of hyperglycemia-augmented damage are the development of postischemic seizures and pannecrosis in the substantia nigra pars reticulata.[5–7] The worsening of the outcome is assumed to be related to the accumulation of lactate plus H^+, produced by the increased amount of glucose anaerobically metabolized.[8] In a previous study we have shown that among the stress hormones and alternative energy substrates investigated only a decrease in the plasma levels of ketone bodies could be responsible for the exaggerated brain damage in hyperglycemic animals.[9]

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Supported by the Swedish Medical Research Council (Grant No. 14X-00263), the National Institutes of Health of the United States Public Health Service (Grant No. 5.ROI NS-07838), the Medical Faculty of Lund University, the Margarethahemmet Society, the Samariten Foundation, the Folke Bernadotte Foundation, the Linnéa and Josef Carlsson Foundation, and "Förenade Liv" Mutual Group Life Insurance.

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Received September 26, 1991; accepted December 13, 1991.

The two rate-limiting factors for access of ketone bodies to the brain are the blood concentrations and the blood–brain barrier.[10–12] Transport across the barrier occurs via a monocarboxylic acid carrier, which appears to be modulated during different physiological conditions (e.g., fasting, diabetes, and the suckling period).[10,13–15] Although the influence of cerebral hypoxia/ischemia on the two rate-limiting factors is not clear, survival after hypoxia was improved in the presence of elevated plasma concentrations of ketone bodies.[16] Furthermore, in vitro studies[17] showed preferential ketone body utilization by rat brain during hypoxia. The adverse effects of decreased ketogenesis on outcome after cerebral insults have recently been supported by studies on 7-day-old hypoxic/ischemic rats[18] and brain trauma patients.[19]

It was previously shown that treatment with 1,3-butanediol (BD), a synthetic lipophilic β-hydroxybutyrate (β-HB) precursor, affords cerebral protection in experimental models of hypoxia and hypoxia/ischemia. Administered BD is metabolized in the liver by alcohol dehydrogenase and aldehyde dehydrogenase to β-HB and acetoacetate (AcAc),[20] which are then released to the blood and available for the brain. BD-induced hyperketonemia caused an increase in survival time in mice exposed to hypoxia[21] and rats exposed to hypoxia/ischemia.[22] However, motor performance was not improved by BD following severe forebrain ischemia,[23] and a similar elevation of the blood concentration of ketone bodies by intravenous infusion of β-HB failed to

afford cerebral protection.[24] In addition, other known effects of BD such as changes in blood glucagon, insulin, and glucose levels were not found to correlate with the protective effect.[24] So far, the mechanisms by which BD exerts its protective effect remain unknown. Nevertheless, Marie et al[25] showed that BD ameliorates ischemic brain damage, accompanied by a reduced brain lactate accumulation and a reduced diminution of high-energy metabolite concentrations after transient forebrain ischemia in rats. Roucher et al[26] extended the observation to the recovery period, showing a faster postischemic recovery of all metabolic variables in animals receiving BD.

The aim of the present study was to find out whether BD also affords protection against transient forebrain ischemia induced under hyperglycemic conditions and whether it alters the development of postischemic seizures.

Materials and Methods

Forty-nine adult male Wistar rats weighing 260–375 g of an SPF strain (Møllegaard's Breeding Center, Copenhagen, Denmark) were used. The animals were housed in Macrolon cages and exposed to a 12-hour day and night cycle. Before ischemia the rats were fasted overnight but allowed water ad libitum. As extensively described earlier,[9] anesthesia was induced with 3.5% isoflurane and 70% N_2O in O_2, whereafter the animal was intubated and connected to a respirator. A central venous catheter, tail venous and arterial catheters, needle electrodes for electroencephalogram (EEG) recording, and temperature probes were inserted. Strings were placed around each common carotid artery. Thereafter 50 units heparin was given intravenously followed by 25 or 50 mmol · kg^{-1} body wt (2.26 or 4.52 ml · kg^{-1} body wt) BD (Aldrich-Chemie, Steinheim, FRG) or 2.26 ml · kg^{-1} body wt 0.9% NaCl intravenously. During a steady-state period lasting 30 minutes, hyperglycemia was attained by infusing a glucose load (about 1 g · kg^{-1} body wt in 5 minutes) followed by a slower infusion (0.04 g · kg^{-1} · min^{-1}) of 25% glucose. After the steady-state period, the glucose and isoflurane administrations were discontinued and ischemia was induced by central venous exsanguination followed by bilateral carotid artery clamping. Blood pressure was maintained around 50 mm Hg during the ischemic period. After 10 minutes of ischemia, brain circulation was restored by removal of the carotid clamps, reinfusion of blood, and an intravenous injection of 0.5 ml of 0.6 M sodium bicarbonate solution. The rats were extubated after 15–30 minutes. During the steady-state and early recirculation periods, the blood pressure was continuously recorded and temperature, Pa_{O_2}, Pa_{CO_2}, and pH were controlled. The procedure was approved by the Ethical Committee for Laboratory Animal Experiments at the University of Lund.

Following 15–16 hours of recovery the rats were perfusion-fixed with phosphate buffered formaldehyde[27]; the brains were then prepared for light microscopic evaluation.[28] The percent damaged neurons was estimated in the parietal cortex, the subiculum, the hippocampus CA1 region, the dentate hilar region, and the lateral reticular, medial ventroposterior, and lateral ventroposterior thalamic nuclei; the percent damaged area (in transsections) was estimated in the caudoputamen, cingulate cortex, and substantia nigra pars reticulata. Grade 0 represents no observable histopathologic changes, while grades 1, 2, and 3 represent 1–5%, 6–50%, and 51–100% damage, respectively.

For brain metabolite analysis the brains were frozen[29] before or at the end of the ischemic period. Concentrations of phosphocreatine, adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, glucose, glycogen, and lactate were analyzed fluorometrically.[30] Blood concentrations of glucose, lactate, β-HB, and AcAc were measured using slight modifications of available enzymatic methods.[31,32]

There were nine groups, two for clinical observation of seizure development and survival (Clin BD and Clin NaCl), three for histopathologic evaluation (Histo BD 25, Histo BD 50, and Histo NaCl), and four for brain metabolite analysis (Met BD sham, Met BD isch, Met NaCl sham, and Met NaCl isch). Rats in all BD-treated groups received 25 mmol · kg^{-1} body wt BD except those in the Histo BD 50 group, which received 50 mmol · kg^{-1} body wt BD. The differences in physiological parameters and blood and brain tissue metabolite levels were compared statistically using analysis of variance followed by Scheffé's test. The differences in the morphological data obtained were analyzed nonparametrically using Kruskal-Wallis one-way analysis of variance by ranks followed by the Mann-Whitney *U* test.

Results

All rats that received BD showed an instant decrease in mean arterial blood pressure (MABP). In rats given 25 mmol · kg^{-1} BD this drop was not to below 75 mm Hg and was very brief. Among the animals that received 50 mmol · kg^{-1} BD the decrease was more profound, and in one rat MABP reached 40 mm Hg, whereafter it gradually increased to low-normal values during the steady-state period. Furthermore, MABP was lower after ischemia in this group than in saline-treated animals (Table 1). Some of the BD-treated rats developed an abnormal EEG. Of the 22 rats that received the lower dose of BD eight showed progressively a somewhat attenuated EEG curve with low voltage and slow activity during the steady-state period; in one animal the attenuated curve was preceded by a period of high-voltage EEG waves. All four rats that received 50 mmol · kg^{-1} BD showed a depression of EEG activity. In two of the animals this EEG abnormality appeared more profound, and in one the EEG recording showed a transient burst-suppression pattern. Importantly, the observed EEG abnormalities did not correlate with the transient decrease in blood pressure because they appeared when the blood pressure was steadily increasing or had already normalized. All rats receiving BD also showed minor hemolysis. Skull temperature was carefully adjusted to and kept at 37.2±0.2°C throughout the experiment. Blood pH appeared lower in the BD-treated groups, but this acidosis was mainly of metabolic origin. Otherwise the physiological variables were very similar in the different groups.

In the control clinical group (Clin NaCl) one of six rats showed transient minor seizure activity after 7 hours of recovery, but no rats developed early seizures. In the Clin BD group two of six animals showed

TABLE 1. Physiological Variables Measured Before and After Hyperglycemic Cerebral Ischemia in Rats

		Variable										
			MABP (mm Hg)		$PaCO_2$ (mm Hg)		PaO_2 (mm Hg)		pH		Base excess (meq · l^{-1})	
Group	*n*	Weight (g)	Before	After	Before	After	Before	After	Before	After	Before	After
Clin BD	6	309±35	109±15	120±8	36±2	38±3	129±16	125±20	7.40±0.03	7.33±0.02*	−1.9±1.8	−5.9±1.9†
Clin NaCl	6	305±26	113±22	138±6	35±2	38±3	119±13	109±11	7.44±0.03	7.42±0.07	0.6±1.2	0.3±3.8
Histo BD 25	8	304±30	114±20	134±12	36±2	39±4	121±9	114±35	7.40±0.02	7.37±0.05	−1.8±0.9	−3.1±2.3
Histo BD 50	4	363±16	96±19	114±9†	35±2	37±3	125±19	125±14	7.41±0.04	7.36±0.03	−1.3±2.4	−4.3±1.8†
Histo NaCl	8	314±35	118±23	138±16	36±2	37±4	130±9	103±29	7.43±0.03	7.43±0.02	0.4±2.0	1.4±2.1
Met BD sham	4	305±23	123±37	...	35±1	...	131±11	...	7.39±0.02	...	−2.8±1.0	...
Met BD isch	4	305±34	119±22	...	36±1	...	128±13	...	7.39±0.04	...	−2.3±2.0	...
Met NaCl sham	5	304±30	105±16	...	38±5	...	119±11	...	7.41±0.02	...	−0.2±3.5	...
Met NaCl isch	4	310±15	110±25	...	38±3	...	124±13	...	7.41±0.04	...	−0.3±1.7	...

Blood gases, pH, and base excess were measured 5 minutes before and 5 minutes after ischemia. Mean arterial blood pressure (MABP) was recorded when isoflurane administration was discontinued and after 5 minutes of recirculation. Values represent mean±SD.
*$p<0.01$ and †$p<0.05$ different from measurement in corresponding NaCl group by Scheffé's test.

transient early postischemic seizures with onset after about 1½ hours of recovery. In both clinical groups the onset of late fatal seizures occurred between 15 and 21 hours of recirculation, and all rats died within a few hours except one animal in the Clin NaCl group, which died between 30 and 42 hours of recovery. Interestingly, in the morphology groups (Histo BD 25, Histo BD 50, and Histo NaCl) no animals developed early seizures. However, shortly before perfusion-fixation after 15–16 hours of recovery four of eight and two of four rats in the Histo BD 25 and Histo BD 50 groups, respectively, showed running seizures, while no animals in the Histo

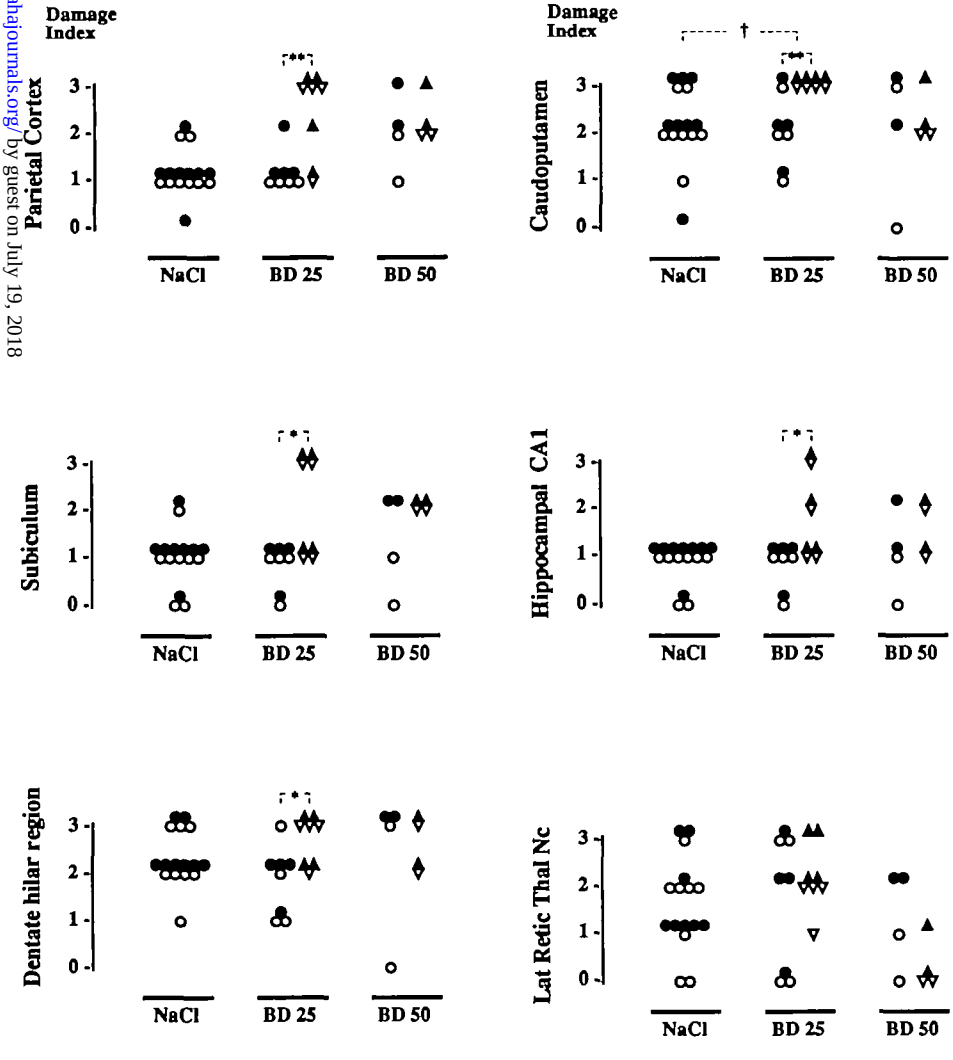

FIGURE 1. *Scatterplots of ischemic damage to six brain regions normally showing selective vulnerability after 10 minutes of ischemia in fasted rats. Lat Retic Thal Nc, lateral reticular thalamic nuclei. Animals were treated with saline (NaCl), 25 mmol · kg^{-1} body wt butanediol (BD 25), or 50 mmol · kg^{-1} body wt butanediol (BD 50). Open symbols represent left and filled symbols right hemisphere. Hemispheres belonging to rats that convulsed before perfusion-fixation are shown by triangles.* †$p<0.05$ *different from NaCl group by Mann-Whitney* U *test.* *$p<0.05$ *and* **$p<0.01$ *difference between convulsing and nonconvulsing animals.*

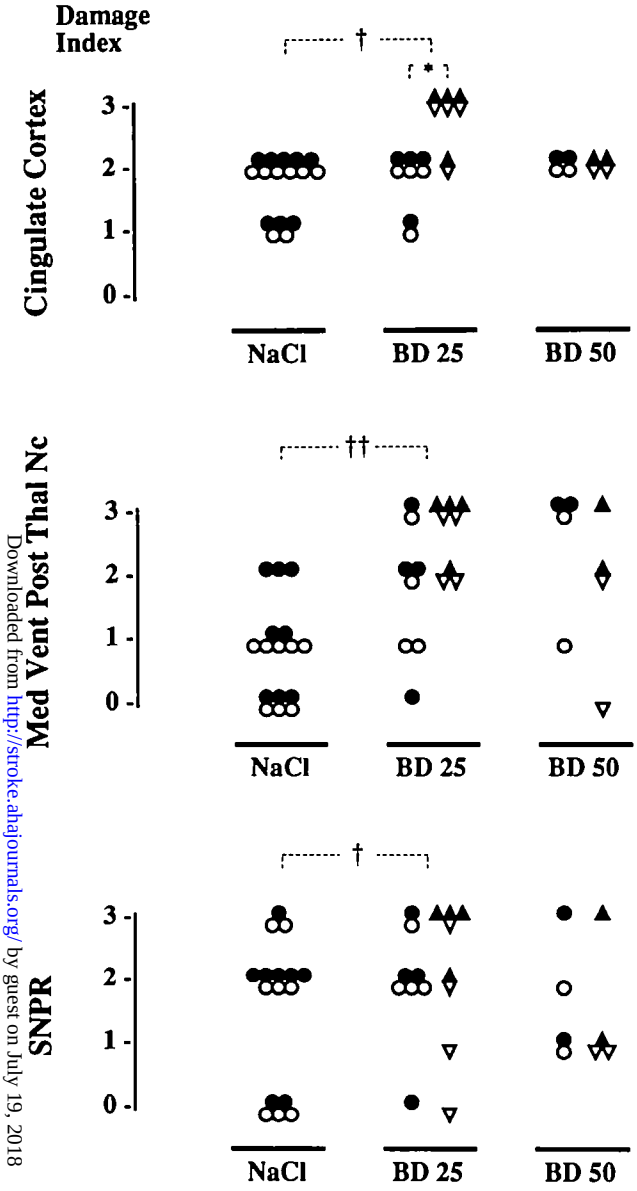

FIGURE 2. *Scatterplots of ischemic damage to three brain regions specifically vulnerable to complicating hyperglycemia in rats. Med Vent Post Thal Nc, medial ventroposterior thalamic nuclei; SNPR, substantia nigra pars reticulata. Animals were treated with saline (NaCl), 25 mmol · kg^{-1} body wt butanediol (BD 25), or 50 mmol · kg^{-1} body wt butanediol (BD 50). Open symbols represent left and filled symbols right hemisphere. Hemispheres belonging to rats that convulsed before perfusion-fixation are shown by triangles.* †p<*0.05 and* ††p<*0.01 different from NaCl group by Mann-Whitney* U *test. *Difference* (p<*0.05) between convulsing and nonconvulsing animals.*

NaCl group showed any similar convulsive activity. After ischemia the BD-treated rats appeared more depressed than the NaCl-treated control rats. In particular, the animals in the Histo BD 50 group showed behavior impairment; all four rats remained lying on their sides after the ischemic insult. Usually, almost all animals attain a prone position shortly after the ischemic period.

The morphological damage (Figures 1 and 2) found in the NaCl-infused rats after ischemia complicated by hyperglycemia showed a pattern and degree of damage similar to that reported earlier.[28,33] Preischemic treatment with 25 mmol · kg^{-1} BD adversely affected the outcome. However, before they were killed four of eight rats treated with 25 mmol · kg^{-1} BD showed seizure activity, which could have aggravated the damage incurred. When these rats were excluded, the aggravation of brain damage in the BD-treated rats compared with the animals that received NaCl was restricted to the medial ventroposterior thalamic nucleus. Treatment with the higher dose of BD appeared to give similar results. In conclusion, BD did not ameliorate damage in any brain structure observed.

TABLE 2. **Blood Levels of Glucose, Lactate, AcAc, β-HB, and AcAc/β-HB Ratio Under Hyperglycemic Conditions Before and After 10 Minutes of Incomplete Cerebral Ischemia in Rats**

	Group		
Metabolite	NaCl (n=13–19)	BD 25 (n=16–19)	BD 50 (n=3 or 4)
Glucose			
Before	12.9±1.6	13.3±1.4	13.5±0.6
After	11.3±1.7	12.3±2.0	12.6±1.2
Lactate			
Before	3.3±1.3	2.4±0.7*	2.0±0.6
After	6.4±1.3†	5.8±1.9†	4.8±1.2‡
AcAc			
Before	0.19±0.09	0.55±0.14§	0.50±0.05§
After	0.08±0.07	0.13±0.10†	0.12±0.08†
Before−after	0.10±0.11	0.40±0.09§	0.35±0.08§
β-HB			
Before	0.15±0.08	0.64±0.29§	0.74±0.13§
After	0.23±0.07	1.00±0.28†§	0.98±0.32§
Before−after	−0.07±0.07	−0.36±0.20§	−0.34±0.19§
AcAc/β-HB ratio			
Before	1.69±1.39	1.05±0.82	0.68±0.06
After	0.39±0.35†	0.13±0.11*‡	0.12±0.07

AcAc, acetoacetate; β-HB, β-hydroxybutyrate. Values are expressed in μmol · ml^{-1} and represent mean±SD.

*p<0.05 and §p<0.01 different from values in NaCl group by Scheffé's test.

†p<0.01 and ‡p<0.05 different from corresponding preischemic value by Scheffé's test.

The blood analysis showed no differences between the different groups receiving 25 mmol · kg^{-1} BD or between the different NaCl-injected groups. Therefore, these groups were pooled and analyzed together. BD-treated rats showed a significantly (p<0.05) lower preischemic but not postischemic level of lactate than NaCl-treated animals (Table 2). The concentration of AcAc decreased and that of β-HB increased during the ischemic period in all groups. Therefore, no difference in the total amount of ketone bodies was observed after compared with before ischemia.

Ketone body levels in brain tissue were low (Table 3). The concentration of AcAc decreased while that of β-HB increased during ischemia in BD-treated rats. Concentrations of the other brain energy metabolites showed no differences between NaCl- and BD-treated animals and changed during ischemia in a fashion similar to that previously reported.[7]

TABLE 3. Brain Levels of Energy Metabolites and Ketone Bodies Under Hyperglycemic Conditions Before and After 10 Minutes of Cerebral Ischemia in Rats

	Before ischemia		After ischemia	
	NaCl (n=5)	BD 25 (n=4)	NaCl (n=4)	BD 25 (n=4)
Glucose	5.82±0.62	5.92±0.72	0.28±0.18*	0.43±0.08*
Adenosine triphosphate	2.90±0.11	2.89±0.09	0.17±0.08*	0.32±0.19*
Phosphocreatine	4.83±0.32	4.65±0.16	0.18±0.15*	0.28±0.13*
Adenosine diphosphate	0.26±0.02	0.26±0.02	0.54±0.11*	0.68±0.05*
Adenosine monophosphate	0.08±0.03	0.10±0.05	1.71±0.10*	1.68±0.16*
Lactate	2.46±0.92	2.89±0.98	19.56±2.18*	21.83±1.37*
Acetoacetate	0.031±0.018	0.061±0.016	0.031±0.010	0.014±0.018†
β-Hydroxybutyrate	0.004±0.008	0.025±0.027	0.029±0.019	0.172±0.107†‡

Values are expressed in $\mu mol \cdot g^{-1}$ and represent mean±SD.
*$p<0.01$ and †$p<0.05$ different from corresponding preischemic value by Scheffé's test.
‡$p<0.01$ different from postischemic value in NaCl group by Scheffé's test.

Discussion

As stated above, substantial evidence exists that BD induces protection in models of hypoxia and hypoxia/ischemia,[21,22,25,26,34] but the mechanisms remain unknown. The decrease in brain lactate concentration after 30 minutes of ischemia in fed, BD-treated animals observed by Marie et al[25] could account for the beneficial effect. Since the difference in outcome following ischemia in normoglycemic and hyperglycemic animals is partly assumed to be related to lactate accumulation,[8] the metabolism of brain ketone bodies and their effect on the glycolytic pathway appear vital. Importantly, when ketone bodies provide an alternative energy substrate for brain metabolism, this requires the consumption of oxygen but does not involve the production of lactate. The enzymes of ketone body utilization in the brain are present in excess.[35] Once within the brain the ketones are quickly and preferentially metabolized yielding acetylcoenzyme A. In nonischemic brain ketone bodies reduce the glycolytic rate, probably due to inhibition of phosphofructokinase activity.[13,36] However, increased utilization of ketone bodies requires glucose metabolism, and in vitro glucose appears to accelerate the use of AcAc.[37] Ketone body utilization by the intact brain also appears to be linked to an increased lactate release to blood.[12,14] However, whether these suggested beneficial effects supplying energy without lactate production, inhibiting glycolysis, and increasing lactate efflux from brain tissue operate during ischemia is not known. We found a preischemic decrease in the blood concentration of lactate in BD-treated rats. The change found in the blood AcAc/β-HB concentration ratio would, however, suggest an increase in the blood lactate level, provided that the pyruvate concentration was unchanged.[38] Importantly, the preischemic decrease in the blood lactate level was not accompanied by a decrease in the brain lactate level. This indicates that no substantial efflux of lactate from the brain with a simultaneous influx of ketone bodies occurred.

It is possible that the decrease in the brain lactate content after ischemia in mild hyperglycemia (fed animals),[25] suggesting an inhibitory effect by ketone bodies on the glycolytic pathway,[13,36] could have been counteracted by the higher blood glucose concentration present in our study. However, the levels of lactate after ischemia in brain tissue from saline-treated fed animals[25] and hyperglycemic (control) animals in our study were similar. This indicates similar amounts of available glucose during ischemia in the two studies.

An alternative explanation for our failure to show any protection could be that the increase in blood β-HB levels was only about 50% as much as previously reported,[25] both before and after 10 minutes of ischemia. This is most likely explained by the hyperglycemia-induced increase in blood levels of insulin,[9] a major regulator of ketone body production from fatty acids in the liver. Also, the intraperitoneal route of administration of BD chosen by Marie et al[25] may have caused higher blood levels by making BD more directly available for conversion to β-HB and AcAc in the liver. We chose the intravenous route because of the possibility that BD may act directly on the brain. BD, like other alcohols, diffuses passively across the blood–brain barrier.[39] It has been speculated that once within the brain, BD is metabolized to β-HB. The required enzymes are available in brain tissue,[35,40] but at exceedingly low activities. Neither prolonged fasting nor a decrease in pH could induce increased enzyme activity.[40] A substantial conversion of BD to β-HB therefore seems very unlikely.[35,39] However, BD could exert an alternative, still unknown, direct protective effect on the brain. Importantly, we achieved a significant ($p<0.01$) increase in the blood concentration of ketone bodies and an EEG change in about half of the BD-treated rats, indicating that the administered BD reached its suggested targets without causing any protective effect.

Apart from the lack of any histopathologic protection by BD, no change in the development of fatal postischemic seizures was observed. It might have been expected that BD, as an alcohol, would afford some anticonvulsive effect. Furthermore, when BD acts as a precursor to ketone bodies it induces biochemical changes similar to those found with a ketogenic diet, a well-known anticonvulsive therapy.[41,42] However, we found no anticonvulsive effect. In the animals intended for the morphology study, BD-treated rats seemed to convulse earlier than NaCl-treated rats. This supports the lack of an anticonvulsive effect, and in fact BD worsened the histopathologic outcome. Since Marie et al[25] used mild hyperglycemia (fed rats), they also encountered problems with seizure activity. Whether the

rats included in their morphology study sustained seizure episodes but survived is not clear from their data. Our results showing increased damage induced by seizures during the postischemic period confirm previous findings[33] and emphasize the importance of taking into account seizure episodes during the recovery period when evaluating the morphological outcome.

We have previously speculated that a lower availability of ketone bodies could be responsible for the difference in outcome caused by ischemic brain damage in normoglycemic and hyperglycemic animals.[9] The results from our present study do not support this suggestion because the threefold to fourfold increase in the blood level of ketone bodies induced by BD caused no improvement but, if anything, seemed to worsen the outcome.

Acknowledgments

The authors gratefully acknowledge the skilled technical assistance of Lena Evergren, Lille-Mor Lindeström, Barbro Asplund, and Lena Sjöberg.

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Stroke. 1992;23:719-724
doi: 10.1161/01.STR.23.5.719
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628