Alterations in Brain Glucose Utilization Accompanying Elevations in Blood Ethanol and Acetate Concentrations in the Rat

Robert J. Pawlosky, **Yoshihiro Kashiwaya**, **Shireesh Srivastava**, **Michael T. King**, **Calvin Crutchfield**, **Nora Volkow**, **George Kunos**, **Ting-Kai Li**, and **Richard L. Veech**
Laboratory of Metabolic Control (RJP, YK, SS, MTK, CC, RLV) and Laboratory of Physiological Studies, National Institute on Alcoholism and Alcohol Abuse (GK), NIH, DHHS, Bethesda, Maryland; Department of Psychiatry and Behavioral Science, School of Medicine Duke University (TKL), Durham, North Carolina; and National Institute on Drug Abuse (NV), NIH, DHHS, Bethesda, Maryland

Abstract

Background—Previous studies in humans have shown that alcohol consumption decreased the rate of brain glucose utilization. We investigated whether the major metabolite of ethanol, acetate, could account for this observation by providing an alternate to glucose as an energy substrate for brain and the metabolic consequences of that shift.

Methods—Rats were infused with solutions of sodium acetate, ethanol, or saline containing ^{13}C-2-glucose as a tracer elevating the blood ethanol (BEC) and blood acetate (BAcC) concentrations. After an hour, blood was sampled and the brains of animals were removed by freeze blowing. Tissue samples were analyzed for the intermediates of glucose metabolism, Krebs' cycle, acyl-coenzyme A (CoA) compounds, and amino acids.

Results—Mean peak BEC and BAcC were approximately 25 and 0.8 mM, respectively, in ethanol-infused animals. Peak blood BAcC increased to 12 mM in acetate-infused animals. Both ethanol and acetate infused animals had a lower uptake of ^{13}C-glucose into the brain compared to controls and the concentration of brain ^{13}C-glucose-6-phosphate varied inversely with the BAcC. There were higher concentrations of brain malonyl-CoA and somewhat lower levels of free Mg^{2+} in ethanol-treated animals compared to saline controls. In acetate-infused animals the concentrations of brain lactate, α-ketoglutarate, and fumarate were higher. Moreover, the free cytosolic $[NAD^+]/[NADH]$ was lower, the free mitochondrial $[NAD^+]/[NADH]$ and $[CoQ]/[CoQH_2]$ were oxidized and the $\Delta G'$ of ATP lowered by acetate infusion from -61.4 kJ to -59.9 kJ/mol.

Conclusions—Animals with elevated levels of blood ethanol or acetate had decreased ^{13}C-glucose uptake into the brain. In acetate-infused animals elevated BAcC were associated with a decrease in ^{13}C-glucose phosphorylation. The co-ordinate decrease in free cytosolic NAD, oxidation of mitochondrial NAD and Q couples and the decrease in $\Delta G'$ of ATP was similar to administration of uncoupling agents indicating that the metabolism of acetate in brain caused the mitochondrial voltage dependent pore to form.

Keywords

Acetate; Brain; Energy Metabolism; ^{13}C-Glucose; $\Delta G'$ ATP

The brain accounts for 20% of the body's O_2 consumption at rest. While under normal resting conditions, the brain's energy needs are supplied by glucose, during prolonged fasting 60% of the brain's energy requirements are supplied by ketone bodies (Owen et al., 1967). Volkow and co-workers have shown that ethanol consumption lowered the rate of brain glucose utilization in humans (Volkow et al., 2006; Wang et al., 2003). Since acetate is the major product of liver metabolism of ethanol, we examined whether elevated blood acetate concentrations (BAcC) in laboratory rats could account for a decrease in brain glucose utilization observed in human subjects consuming alcohol.

It has been long recognized that the brain can metabolize acetate specifically in glial cells where it produces glutamine which is then exported to neurons (Berl and Clarke, 1984). Recent studies using ^{13}C MRS have shown that acetate is significantly utilized in brain metabolism (Deelchand et al., 2009). Acetate thus shares with the other monocarboxylates, ketone bodies and lactate, the ability to be transported into brain and to be used in place of glucose (Simpson et al., 2007). Since the usual precursor of elevated blood acetate is ethanol, we studied the effects of both ethanol and acetate on ^{13}C-glucose uptake in brain. Moreover, since differing substrates traversing the Krebs citric acid cycle have different inherent enthalpic values, we examined the effects of ethanol and acetate on brain energy metabolism.

Animals received a bolus of a solution of sodium acetate, ethanol or saline (containing ^{13}C-2-glucose as a tracer) followed by a constant infusion of the same solution for an hour. After which time, blood was sampled, animals were killed, and the brains were analyzed for glycolytic, TCA cycle, and other metabolites. The concentrations of the metabolic intermediates were used to calculate cytosolic and mitochondrial redox potentials and the ΔG of ATP hydrolysis.

MATERIAL AND METHODS

Animal Procedures

All experiments were reviewed and approved by the Animal Care and Use Committee of NIAAA, NIH. Male Wistar rats weighing 280 to 310 g (n = 6 to 8 per group) were obtained from Charles River Laboratories, Wilmington, MA, and divided into 3 groups. Twenty four hours prior to infusion the jugular veins of animals were cannulated using polyethylene tubing (PE-50). Animals recovered from surgery for 24 hours and were maintained on NIH 31 rat diet. Prior to infusions animals were fasted overnight.

Infusions

Each animal received a bolus (0.08 ml/100 g body weight) of 1 of 3 solutions: saline-control (PBS, phosphate buffered saline with 0.73 mg/ml ^{13}C-2 glucose), sodium-acetate (2 M in PBS + 0.73 mg/ml ^{13}C-2 glucose), and ethanol (4 Min PBS+0.73 mg/ml ^{13}C-2 glucose). The bolus dose was followed by infusion of solutions at a rate of 1.4 ml/h/100 g body weight. Following infusions, blood samples were collected and brain removed by freeze blowing (Veech et al., 1973) and stored at −80°C until analyzed.

Analytical Procedures

Krebs cycle intermediates were measured using gas chromatography-mass spectrometry (GC-MS). The coenzyme A's (CoA) and nitrogenousmetabolites were measured using capillary electrophoresis-mass spectrometry (CE-MS). The number of samples analyzed for each metabolite varied from 5 to 8. All other metabolites were measured enzymatically as described previously (Sato et al., 1995).

Sample Preparation for GC-MS and CE-MS Analyses

Procedures for the determination of citrate, isocitrate, α-ketoglutarate, succinate, fumarate, and malate by GC-MS are described below. The organic acids were analyzed as their tertiary butyl dimethylsilyl ether derivatives (TBDMS) using GC-MS in the electron impact mode and quantified using the ^{13}C-labeled internal standards for each analyte. The N-methyl-N-(tert-butylmethylsilyl) trifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylchlorosilane (TBDMCS) reagent was purchased from Pierce Chemical Co. (Rockford, IL) and the ^{13}C-labeled organic acids, citrate, succinate, fumarate, and malate were procured from Sigma Chemical Co. (St Louis, MO). The ^{13}C-labeled isocitrate and α-ketoglutarate were prepared in the laboratory according to known procedures (Barman, 1969; Ehrlich and Colman, 1987).

Brain samples for GC-MS analysis were prepared by perchloric acid extraction as previously described (Veech et al., 1973). The ^{13}C-labeled internal standards were added in twofold excess of the concentrations of the individual analytes in brain tissue to the neutralized PCA extracts. Extracts (50 μl) were evaporated under a stream of nitrogen to dryness. Samples were immediately reacted with 5 μl of the sylilating reagent in 15 μl of acetonitrile in 1.5 ml screw capped glass vials and heated to 60°C for 5 minutes. Samples were analyzed on an Agilent 5973 quadrupole GC-MS (Agilent, Wilmington, DE). One μl of the sample solutions was injected onto a 250 μm × 30 m capillary DB-1 (Agilent) column in the splitless injection mode using helium as the carrier gas. The injector temperature was set at 270°C and the transfer line at 280°C. The GC oven temperature was programmed from 80 to 325°C at 15°C/min. The mass spectrometer was operated in the electron impact mode (70 eV) and the quadrupole mass analyzer scanned for ions which corresponded to a loss of 15 mass units (-CH$_3$) from the molecular ion and the base peak of each analyte and its corresponding ^{13}C-labeled internal standard using selected ion monitoring. Analytes eluted the column between 10 and 20 minutes following injection and the ratio of peak area counts of the ^{13}C-labeled internal standard to that of the analyte were used to quantify its concentration.

Standards for CE-MS Analysis of CoA, Acetyl CoA, Malonyl CoA, Succinyl CoA, Glutamate, Aspartate

The labeled internal standards, ^{13}C-glutamate, ^{13}C-aspartate, ^{13}C-acetyl CoA, and ^{13}C-malonyl CoA were purchased from Sigma Chemical Co. (St Louis, MO). ^{13}C-succinyl CoA was synthesized in the laboratory.

Sample Extraction for CE-MS Analysis

Brain tissue samples were extracted using a modified chloroform-methanol extraction procedure (Soga et al., 2002). Approximately 50 mg of frozen whole brain tissue was added to a frozen (−80°C) solution of water:chloroform:methanol (300:400:200 μl) containing the labeled internal standards in twofold excess in concentration of the analyte in tissue in 2 ml polypropylene screw capped tubes with 40 to 50 small glass beads. Samples were shaken onMini Bead Beater (Biopsec Products, Bartlesville, OK) for 30 seconds (2×) until samples were homogeneous. Samples were placed on ice until centrifugation at 4°C in a Sorvall benchtop centrifuge at a speed of 10.7 × g for 10 minutes to allow the chloroform and water layers to separate. The water layer was removed and filtered through a protein filter centrifuge tube, MW cut off 10,000 Da at 90 × g. The filtrate (about 300 μl)was used in CE-MS analysis.

CE-MS Analysis

Sample extracts were analyzed on an Agilent capillary electrophoresis-ion trap mass spectrometer (Ultra) using an Agilent 1100 series binary pump to deliver a make-up flow of 50 mM ammonium acetate in methanol:water (50:50) to the electrospray ionization tip (Agilent). Samples were ionized in either the positive or negative ion ionization modes. Anions

and cations were analyzed on $50\,\mu m \times 100$ cm dynamically cationic or anionic coated capillary columns using either positive (+30 kV) or negative (−30 kV) electro-kinetic injections. The capillary column was cooled to 20°C using the thermostatic controlled capillary cassette and the electrospray needle was set orthogonal to the mass spectrometer inlet. The CE electrolyte used for separation was 50 mM ammonium acetate (pH 9.0) and the column was flushed successively between runs with acetic acid (pH 3.4) and ammonium acetate before injection. The applied voltage was set at −30 kV. The binary pump was set to deliver $8\,\mu l$ of 5 mM ammonium acetate in water:methanol (50:50) as a sheath liquid to the capillary tip. The mass spectrometer was scanned throughout various mass ranges to accommodate the analysis for the amino acids (150 to 300 amu) or the CoA compounds (700 to 1100 amu). Analytes were quantified using the ratio of peak area counts of the ^{13}C-labeled internal standard pseudo molecular ion to that of the analyte.

Calculations

The calculation of free $[Mg^{2+}]$, phosphorylation potential, free cytosolic [ADP], [AMP], [Pi], [oxaloacetate], the ΔG of ATP hydrolysis, cytosolic and mitochondrial redox states were calculated as described previously (Veech et al., 1979, 1994; Veloso et al., 1973).

Statistics

The results are presented as: means ± SEM. A paired *t*-test with the Bonferroni adjustment for multiple group comparisons were used to determine the level of significance between means of the various experimentally determined intermediates, the derived redox couples and nucleotides between each of the 3 groups. Regression analysis was carried out using StatView statistical software from Adept Scientific (Letchworth Garden City, U.K.) and significance was determined using ANOVA. A *p*-value<0.05 was considered significant.

RESULTS

Blood Glucose, Acetate, Ethanol Concentrations

Animals remained alert during infusion procedures, although ethanol-infused animals were less active compared to animals in the other 2 groups. Fasting blood glucose concentrations were within normal limits for all 3 groups (Table 1). Mean blood ethanol concentrations (*BEC*) increased to 25 mM(range: 8 to 31 mM) an hour after infusion of the ethanol solution. The BAcC increased over baseline values but remained below 1 mM (Fig. 1). As predicted, BAcC in acetate-infused group were elevated to a mean value of 12 mM (range: 2.5 to 20 mM) 1 hour after beginning infusion. Although, mean BEC and BAcC values tended to be proportional to their respective concentrations of the infusate the blood concentration range for both blood ethanol and acetate was variable.

Brain Glycolytic Intermediates

There were no differences in concentrations of brain phosphorylated intermediates, glucose-6-phosphate, di-hydroxy acetone phosphate, or 3-phosphoglycerate between the ethanol or acetate infused animals and the saline infused controls. However, in the brains of the acetate-infused animals the concentration of lactic acid was greater compared to either the ethanol-treated or saline groups (Table 2). Consequently, the brain free cytosolic [NAD$^+$]/[NADH] (determined by lactate and pyruvate concentrations) were more reduced (mean value: 257) compared to either ethanol-treated (mean: 397) or saline (mean: 392) (Table 3). An increase in the concentration of brain lactate in the presence of a more abundant energy substrate, acetate, suggests a possible decrease in glucose utilization. However, the concentration of acetyl-CoA was no different among any of the groups (Table 4). In ethanol-infused animals, both brain lactate concentrations and cytosolic redox potential (Table 3) were no different from

saline-controls, showing that unlike liver where free cytosolic $[NAD^+]/[NADH]$ is markedly reduced by ethanol metabolism, the brain which lacks alcohol dehydrogenase (Veloso et al., 1973) may only metabolize ethanol through cytochrome P450 (Anandatheerthavarada et al., 1993) or catalase pathways (Cohen et al., 1980).

Uptake and Utilization of ^{13}C-Glucose Into the Brain

At the end of the infusion period ^{13}C-enrichments of blood ^{12}C-glucose (uncorrected for naturally occurring C-13 abundance) were similar among the 3 groups (Table 5). In the saline controls, ^{13}C-glucose enrichments had equilibrated across the blood-brain interface (blood: 0.442, brain: 0.461). In comparison, in brain extracts from either the ethanol- and acetate-infused animals the ^{13}C-glucose enrichments tended to be lower (ethanol: 0.367, $p < 0.06$; acetate: 0.295, $p < 0.07$) than in saline-infused controls. Moreover, ^{13}C-glucose enrichments across the blood-brain interface approached but did not reach equilibrium in either ethanol- or acetate-treated animals (Table 5). Displacement from equilibrium was reflected by a more negative Δ value in these groups and suggested an inhibition in glucose transfer (see Table 5 for the Δ value calculation). Consistent with the observation of a decrease in the uptake and utilization of glucose in acetate-infused animals, there was also an inverse association (correlation coefficient, $R = 0.85$, $p < 0.03$) between brain ^{13}C-glucose-6-phosphate concentration and the BAcC (see Fig. 2). However, there was no association between ^{13}C-glucose-6-phosphate and BEC in ethanol-infused animals again demonstrating that the effect on brain glucose 6- phosphate is a result of acetate and not directly from ethanol.

Krebs Cycle Intermediates

Ethanol-infused animals had a small but statistically significant lower concentrations of brain citric acid compared to the saline-infused controls and significantly lower concentrations with respect to the acetate-infused group (Table 4). As reported previously the citrate/isocitrate ratio may be used as a determinant of the concentration of freeMg^{2+} in the system (Veloso et al., 1972). Acetate-infused animals had higher free Mg^{2+} concentrations compared to the saline- or ethanolinfused groups. Ethanol-treated animals had significantly higher concentrations of malonyl CoA than saline-infused animals. In acetate-infused animals there were higher concentrations of fumarate compared to the saline controls and higher amounts of α-ketoglutarate than either the saline- or ethanol-infused animals. However, there were no differences in isocitrate, succinate, succinyl CoA, and malate concentrations in animals from either acetate- or ethanol-infused groups compared to the saline group (Table 4).

Nucleotide and Amino Acid Concentrations

There were several differences in the values of the reduced and oxidized nucleotides (calculated from the concentrations of various metabolic intermediates) in acetate-infused animals compared to either the saline- or ethanol-infused groups (Table 3). Acetate-infused animals had a lower cytosolic free $[NAD^+]/[NADH]$ ratio compared to ethanol-infused animals and a lower but nonsignificant ($p < 0.057$) redox potential compared to the saline controls. Acetate infused animals had an increased free mitochondrial $[NAD^+]/[NADH]$ ratio and an increased free $[CoQ]/[CoQH_2]$ ratio (Table 3). This reduction in the ratio of free cytosolic $[NAD^+]/[NADH]$ in the presence of a more oxidized free mitochondrial redox potential $[NAD^+]/[NADH]$ is an unusual pattern, found only rarely in situations such as the administration of uncoupling agents such as carbonyl cyanide p-trifluoromethoxyphenyl hydrazone (FCCP) (Spry, 1971). Compatible with this inference was also a significant decrease in the energy of ATP hydrolysis in acetate- infused animals compared to either the ethanol-infused or saline-infused group. There were no differences in the concentrations neither of brain glutamate or aspartate nor of their ^{13}C-labeled analogues (Table 6) nor high energy intermediates between any of the groups (Table 7).

DISCUSSION

This is the first study to our knowledge investigating the effects of elevated blood acetate and ethanol concentrations on alterations of brain energetic profiles. Previous studies have investigated the effects of labeled-acetate in supplanting glucose utilization on brain metabolites (Deelchand et al., 2009; Serres et al., 2008; Tyson et al., 2003). Aside from blood acetate derived from the metabolism of ethanol, patients with renal failure undergoing dialysis treatment may also have highly elevated BAcC (Mansell et al., 1979).

Previously, Volkow and coworkers using PET imaging demonstrated that human subjects consuming even low amounts of alcohol had decreased brain glucose uptake (Volkow et al., 2006). Yet, it remains unclear whether this effect is primarily due to blood ethanol concentrations or to a combination of increases in blood ethanol and its metabolite, acetate (Lundquist, 1962).

We found that when rats were infused with solutions of ethanol or sodium acetate, both substances decreased ^{13}C-glucose enrichments in the brains. However, in the present study highly elevated BEC together with increases in BAcC (<1 mM) did not cause a significant decrease in the phosphorylation of ^{13}C-glucose whereas, in acetate-infused animals the decrease in brain ^{13}C-glucose-6-phosphate was proportional to the blood acetate concentration suggesting a probable cause and effect relationship.

Elevations in blood acetate also had widespread effects on brain cytosolic and mitochondrial redox potentials and on the phosphorylation potential. In acetate-infused animals there was a decrease in the free cytosolic $[NAD^+]/[NADH]$ ratio, an increase of the free mitochondrial $[NAD^+]/[NADH]$ ratio, and the free mitochondrial $[CoQ]/[CoQH_2]$, and a decrease in the $\Delta G'$ of ATP hydrolysis. This pattern of changes was also seen with the administration of uncoupling agents (Spry, 1971). However, a more plausible explanation for these observations is that high concentrations of acetate elevated the inorganic pyrophosphate (PPi) levels in the brain. Highly elevated PPi concentrations were previously reported in the liver of rats given intraperitoneal (IP) injections of sodium acetate or other short chain fatty acids (Veech et al., 1986). Elevated acetate concentrations were shown to cause an influx of divalent cations, Ca^{2+} and Mg^{2+} into the liver mitochondria forming precipitates in the presence of 2 mM CaMgPPi. This process of rapid formation of complexes of CaMgPPi within the mitochondria is only compatible with the formation of the mitochondrial permeability transition pore provided that an opening of a calcium ion channel and activation of protein kinase A also occurs (Yamada et al., 1988). Beavis and Garlid demonstrated that depletion of Mg^{2+} from the mitochondria matrix causes the formation of the unregulated mitochondrial permeability transition pore (Beavis and Garlid, 1987). It is likely that the activation of acetate in the brain via,

$$acetate+CoA+ATP \rightarrow acetylCoA+AMP+PPi \tag{1}$$
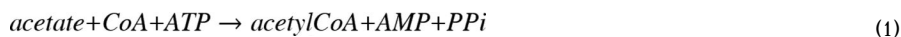
by acetate thiokinase (EC 6.2.1.1) leads to formation of PPi in the matrix depleting free mitochondrial Mg^{2+} by forming CaMgPPi and forming the mitochondrial permeability transition pore in the brain as it does in liver. In the current study the threefold influx of Mg^{2+} into the cytosol of brains of acetate- treated animals is compatible with processes that cause a depletion of free mitochondrial Mg^{2+} through the formation of CaMgPPi complexes. Excitotoxic neuronal death resulting from an overstimulation of glutamate receptors, also is known to cause large influxes of divalent cations into the mitochondria effecting formation of mitochondrial permeability transition pore (Schinder et al., 1996).

Formation of the mitochondrial permeability transition pore, with a necessary destruction of the mitochondrial H^+ gradient would account for the reduction of the cytosolic $[NAD^+]/$ $[NADH]$, the oxidation of the free mitochondrial NAD and CoQ couples and the decrease in the phosphorylation potential observed in the acetate treated group.

This study confirms that high elevations in blood acetate (above 2 mM) alter several metabolic parameters related to energy production in the brain in rats. The metabolism of acetate in the brain decreased the ΔG of ATP hydrolysis whereas high concentrations of blood ethanol with lower concentrations of blood acetate did not alter similar metabolic processes. In man, blood acetate can reach as high a level as 1.5 mM when consuming variable quantities of alcohol (Chen et al., 2009; Lundquist, 1962). It is therefore possible under certain hormonal conditions (Yamada et al., 1988), pore opening could occur during ethanol consumption and its metabolism in man. It is therefore possible that the ability of acetate to form the mitochondrial permeability transition pore, may under certain conditions, account for some ethanol related cerebral pathologies.

References

Anandatheerthavarada HK, Shankar SK, Bhamre S, Boyd MR, Song BJ, Ravindranath V. Induction of brain cytochrome P-450IIE1 by chronic ethanol treatment. Brain Res 1993;601:29–85.

Barman, TE. Enzyme Handbook. Springer-Verlag; Berlin: 1969.

Beavis AD, Garlid KD. The mitochondrial inner membrane anion channel. J Biol Chem 1987;262(31): 15085–15093. [PubMed: 2444594]

Berl, S.; Clarke, DD. Cerebral glutamine/glutamate interrelationships and metabolic compartmentation. In: Haussinger, D.; Sies, H., editors. Glutamine Metabolism in Mammalian Tissues. Springer-Verlag; New York: 1984. p. 150-222.

Chen YC, Peng GS, Tsao TP, Wang MF, Lu RB, Yin SJ. Pharmacokinetic and pharmacodynamic basis for overcoming acetaldehyde-induced adverse reaction in Asian alcoholics, heterozygous for the variant ALDH2*2 gene allele. Pharmacogenet Genomics 2009;19(8):588–599. [PubMed: 19584771]

Cohen G, Sinet PM, Heikkila R. Ethanol oxidation by rat brain in vivo. Alcohol Clin Exp Res 1980;4(4): 366–370. [PubMed: 7004234]

Deelchand DK, Shestov AA, Koski DM, Ug¢urbil K, Henry PG. Acetate transport and utilization in the rat brain. J Neurochem 2009;109(Suppl):46–54. [PubMed: 19393008]

Ehrlich RS, Colman RF. Ionization of isocitrate bound to pig heart NADP+-dependent isocitrate dehydrogenase: 13C NMR study of substrate binding. Biochemistry 1987;26:3461–3466. [PubMed: 3651391]

Lundquist R. Production and utilization of free acetate in man. Nature 1962;193:579–580. [PubMed: 14467396]

Mansell MA, Nunan TO, Laker MF, Boon NA, Wing AJ. Incidence and significance of rising blood acetate levels during hemodialysis. Clin Nephrol 1979;12(1):22–25. [PubMed: 477053]

Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF Jr. Brain metabolism during fasting. J Clin Invest 1967;46(10):1589–1595. [PubMed: 6061736]

Sato K, Kashiwaya Y, Keon CA, Tsuchiya N, King MT, Radda GK, Chance B, Clarke K, Veech RL. Insulin, ketone bodies, and mitochondrial energy transduction. FASEB J 1995;9:651–658. [PubMed: 7768357]

Schinder AF, Olson EC, Spitzer NC, Montal M. Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. J Neurosci 1996;16(19):6125–6133. [PubMed: 8815895]

Serres S, Raffard G, Franconi J-M, Merle M. Close coupling between astrocytic and neuronal metabolisms to fulfill anaplerotic and energy needs in the rat brain. J Cereb Blood Flow Metab 2008;28:712–724. [PubMed: 17940539]

Simpson IA, Carruthers A, Vannucci SJ. Supply and demand in cerebral energy metabolism: the role of nutrient transporters. J Cereb Blood FlowMetab 2007;27(11):1766–1791.

Soga T, Ueno Y, Naraoka H, Ohashi Y, Tomita M, Nishiokat T. Simultaneous determination of anionic intermediates for Bacillus subtilis metabolic pathways by capillary electrophoresis electrospray ionization mass spectrometry. Anal Chem 2002;74:2233–2239. [PubMed: 12038746]

Spry, MS. BSc Thesis: Relationships between the redox state of the pyridine nucleotides, the phosphorylation state of the adenine nucleotides and substrate utilization in animal tissues. St. Anne's College, Oxford University; Oxford, England: 1971.

Tyson RL, Gallagher C, Sutherland GR. 13C-labeled substrates and the cerebral metabolic compartmentalization of acetate and lactate. Brain Res 2003;992:43–52. [PubMed: 14604771]

Veech RL, Gates DN, Crutchfield CW, Gitomer WL, Kashiwaya Y, King MT, Wondergem R. Metabolic hyperpolarization of liver by ethanol: the importance of Mg2+ and H+ in determining impermeant intracellular anionic charge and energy of metabolic reactions. Alcohol Clin Exp Res 1994;18:1040–1056. [PubMed: 7531402]

Veech, RL.; Gitomer, WL.; King, MT.; Balaban, RS.; Costa, JL.; Eanes, ED. The effect of short chain fatty acid administration on hepatic glucose, phosphate, magnesium and calcium metabolism. Myocardial and Skeletal Muscle. In: Nachman, B., editor. Bioenergetics. Plenum Publishing Corp; New York: 1986. p. 617-646.

Veech RL, Harris RL, Veloso D, Veech EH. Freeze-blowing: a new technique for the study of brain in vivo. J Neurochem 1973;20:183–188. [PubMed: 4405707]

Veech RL, Lawson JWR, Cornell NW, Krebs HA. Cytosolic phosphorylation potential. J Biol Chem 1979;254:6538–6547. [PubMed: 36399]

Veloso D, Guynn RW, Oskarsson M, Veech RL. The concentrations of free and bound magnesium in rat tissues. J Biol Chem 1973;248(13):4811–4819. [PubMed: 4718747]

Veloso D, Passonneau JV, Veech RL. The effects of intoxicating doses of ethanol upon intermediary metabolism in rat brain. J Neurochem 1972;19(11):2679–2686. [PubMed: 4673618]

Volkow ND, Wang GJ, Franceschi D, Fowler JS, Thanos PP, Maynard L, Gatley SJ, Wong C, Veech RL, Kunos G, Kai Li T. Low doses of alcohol substantially decrease glucose metabolism in the human brain. Neuroimage 2006;29(1):295–301. [PubMed: 16085426]

Wang GJ, Volkow ND, Fowler JS, Franceschi D, Wong CT, Pappas NR, Netusil N, Zhu W, Felder C, Ma Y. Alcohol intoxication induces greater reductions in brain metabolism in male than in female subjects. Alcohol Clin Exp Res 2003;27(6):909–917. [PubMed: 12824811]

Yamada T, Inoue T, Nishida T, Furuya E, Tagawa K. Hepatic Accumulation of pyrophosphate during acetate metabolism. J Biochem 1988;104:847–850. [PubMed: 2853160]

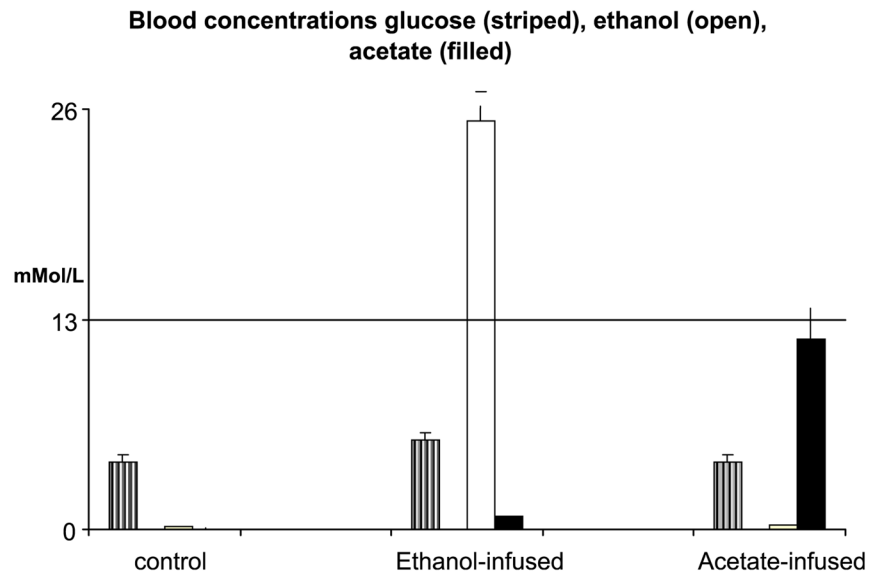**Blood concentrations glucose (striped), ethanol (open), acetate (filled)**

Fig. 1.
Blood glucose, ethanol, and acetate concentrations from rats given bolus doses and infused with solutions of saline (control), sodium acetate (2 M), or ethanol (4 M) in phosphate buffered saline after 1-hour infusion.

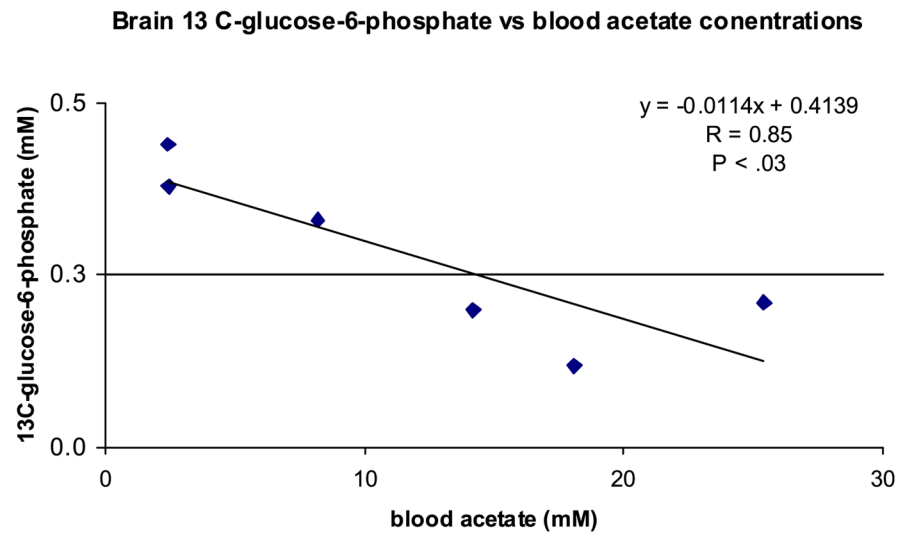**Brain 13 C-glucose-6-phosphate vs blood acetate conentrations**

Fig. 2.
Linear regression of blood acetate concentration versus brain ^{13}C-glucose-6-phosphate in rats infused with sodium acetate (2 M) after 1-hour infusion. Correlation coefficient, $R = 0.85$ and $p < 0.03$ (ANOVA).

Table 1

Blood Glucose, Ethanol, and Acetate Concentrations

	Saline (mM/l)	Ethanol (mM/l)	Acetate (mM/l)
Glucose	4.15 ± 0.21	5.50 ± 0.05	4.17 ± 0.44
Ethanol	0.18 ± 0.03	25.3 ± 1.76[a,b]	0.23 ± 0.08
Acetate	0.04 ± 0.01	0.77 ± 0.06[a]	11.79 ± 1.87[a,b]

Animals infused with solutions of phosphate buffered saline (PBS) ($n = 5$), 4 M ethanol in PBS ($n = 8$), or 2 M sodium acetate in PBS ($n = 6$).

[a] $p < 0.05$ between ethanol- or acetate-infused group and saline.

[b] $p < 0.05$ between ethanol-infused and acetate-infused, paired *t*-test with Bonferroni adjustment for multiple group comparisons.

Table 2

Brain Glycolytic Intermediates

	Saline	Ethanol	Acetate
Glucose-6-phosphate	0.095 ± 0.007	0.086 ± 0.006	0.104 ± 0.012
DHAP	0.027 ± 0.001	0.031 ± 0.004	0.024 ± 0.003
3-Phos-glycerate	0.020 ± 0.002	0.023 ± 0.003	0.021 ± 0.003
Pyruvate	0.094 ± 0.012	0.091 ± 0.011	0.081 ± 0.012
L-Lactate	1.37 ± 0.04	1.29 ± 0.15	$1.77 \pm 0.08^{a,b}$

Animals infused with solutions of phosphate buffered saline (PBS) ($n = 5$), 4 M ethanol in PBS ($n = 8$), or 2 M sodium acetate in PBS ($n = 6$). Values are means in μmole/g wet weight $\pm$ SEM.

[a] $p < 0.05$ between acetate-infused and saline groups.

[b] $p < 0.05$ between acetate- and ethanol-infused groups, paired *t*-test with Bonferroni adjustment for multiple group comparisons.

Table 3

Calculated Free Nucleotide Concentrations and Ratios

	Saline	Ethanol	Acetate
Free [NAD$^+$]/[NADH] cytosol from lactate/pyruvate	392.7 ± 57.3	397.2 ± 36.9	257.3 ± 33.5^b
Free [NAD$^+$]/[NADH] mitochondria from αKG $\times$ NH$_4^+$/Glut	1.03 ± 0.05	1.04 ± 0.05	$1.32 \pm 0.08^{a,b}$
Free [NADP$^+$]/[NADPH] cytosol from αKG $\times$ CO$_2$/IsoCit	0.0130 ± 0.0003	0.0133 ± 0.0010	0.0165 ± 0.0014
Free mitochondrial [CoQ]/[CoQH$_2$] from measured Fum/Succ	0.0047 ± 0.0004	0.0056 ± 0.0006	0.0062 ± 0.0004^a
Free [Mg^{2+}] mM from [Cit]/[Isocit]	0.333 ± 0.129	0.261 ± 0.068	0.750 ± 0.181^b
Phosphorylation potential from kg + g (Eq. 1)/M	$39,315 \pm 5,379$	$43,807 \pm 7,528$	$33,082 \pm 3,992$
$\Delta G'$ ATP kJ/mol	-61.4 ± 0.4	-61.3 ± 0.5	$-59.9 \pm 0.3^{a,b}$
Free [ADP] cytosol μM/g from PCr/Cr	0.029 ± 0.006	0.043 ± 0.016	0.035 ± 0.006
Free [AMP] cytosol μM/g from $K_{myokinase}$	0.0003 ± 0.00008	0.0009 ± 0.0006	0.0006 ± 0.0001

Animals infused with solutions of phosphate buffered saline (PBS) ($n = 5$), 4 M ethanol in PBS ($n = 8$), or 2 M sodium acetate in PBS ($n = 6$). Values are means in μmole/g wet weight $\pm$ SEM. The pH assumed to be 7.2. For methods of calculation, see references Veloso and colleagues (1973) and Veech and colleagues (1979, 1994).

[a] $p < 0.05$ between acetate-infused and saline.

[b] $p < 0.05$ between acetate- and ethanol-infused groups, paired t-test with Bonferroni adjustment for multiple group comparisons.

Table 4

Brain Krebs Cycle and Coenzyme A (CoA) Intermediates

	Saline	Ethanol	Acetate
Citrate	0.286 ± 0.034	0.268 ± 0.023[b]	0.373 ± 0.047
Isocitrate	0.022 ± 0.0005	0.023 ± 0.001	0.022 ± 0.0002
α-Ketoglutarate	0.170 ± 0.004	0.170 ± 0.005	0.201 ± 0.007[a,b]
Succinyl CoA (μM)	3.36 ± 0.47	4.15 ± 0.41	3.54 ± 0.24
Succinate	0.160 ± 0.007	0.145 ± 0.010	0.158 ± 0.008
Fumarate	0.128 ± 0.010	0.133 ± 0.010	0.169 ± 0.013[a]
l-Malate	0.266 ± 0.015	0.252 ± 0.024	0.275 ± 0.034
Calc oxaloacetate	0.0068 ± 0.0002	0.0063 ± 0.0005	0.0089 ± 0.0008[a,b]
Acetyl CoA (μM)	3.40 ± 1.32	3.35 ± 0.31	2.78 ± 0.21
Malonyl CoA (μM)	2.05 ± 0.33	3.15 ± 0.31[a]	2.32 ± 0.15

Animals infused with solutions of phosphate buffered saline (PBS) ($n = 5$), 4 M ethanol in PBS ($n = 8$), or 2 M sodium acetate in PBS ($n = 6$). Values are means in μmole/g wet weight ± SEM. CoA's are given in nmol/g wet weight.

[a] $p < 0.05$ between infused and saline group.

[b] $p < 0.05$ between ethanol-infused and acetate-infused, paired t-test with Bonferroni adjustment for multiple group comparisons.

Table 5

Blood and Brain ^{13}C-Glucose Enrichments and Brain ^{13}C-Glucose-6-Phosphate Concentrations

	Saline	Ethanol	Acetate
Blood ^{13}C/^{12}C-glucose enrichments[a]	0.448 ± 0.023	0.426 ± 0.008	0.360 ± 0.051
Brain ^{13}C/^{12}C-glucose enrichments[a]	0.461 ± 0.059	0.367 ± 0.008	0.295 ± 0.048
Δ (percent difference in blood-brain enrichments)[b]	2.8 ± 1.1	−16.4 ± 3.5[a]	−27.3 ± 7.7[d]
Brain ^{13}C-glucose-6-phosphate[c]	0.091 ± 0.013	0.070 ± 0.016	0.066 ± 0.007[e]

Animals infused with solutions of phosphate buffered saline (PBS) ($n = 5$), 4 M ethanol in PBS ($n = 8$), or 2 M sodium acetate in PBS ($n = 6$). Infusates contained 1.8 mg/ml of ^{13}C-2-glucose as tracer.

[a]Values are glucose ^{13}C/^{12}C enrichments ± SEM.

$$_b\Delta= \left\{ \frac{\frac{^{13}C}{^{12}C}\text{glucose}_{\text{blood}} - \frac{^{13}C}{^{12}C}\text{glucose}_{\text{brain}}}{\frac{^{13}C}{^{12}C}\text{glucose}_{\text{blood}}} \right\} \times 100$$

[c]Values are means in μmole/g wet weight ± SEM.

[d]$p < 0.01$ between ethanol- or acetate-infused animals and saline, paired t-test with Bonferroni adjustment for multiple group comparisons.

[e]$p < 0.03$ between acetate-infused animals and saline controls, ANOVA.

Table 6

Brain Amino Acids

	Saline	Ethanol	Acetate
L-Glutamate	10.59 ± 0.32	10.48 ± 0.29	9.78 ± 0.50
L-Aspartate	2.79 ± 0.11	2.55 ± 0.17	2.85 ± 0.16

Animals infused with solutions of phosphate buffered saline (PBS) ($n = 5$), 4 M ethanol in PBS ($n = 8$), or 2 M sodium acetate in PBS ($n = 6$). Values are means in μmole/g wet weight $\pm$ SEM.

Table 7

Brain High Energy Intermediates

	Saline	Ethanol	Acetate
ATP	2.49 ± 0.173	2.64 ± 0.19	2.39 ± 0.19
P-Creatine	3.30 ± 0.12	3.37 ± 0.11	3.54 ± 0.10

Animals infused with solutions of phosphate buffered saline (PBS) ($n = 5$), 4 M ethanol in PBS ($n = 8$), or 2 M sodium acetate in PBS ($n = 6$). Values are means in μmole/g wet weight ± SEM.