Mechanisms of Increased Brain Glucose and Glycogen after Hydrocortisone: Possible Clinical Significance

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We reported previously that chronic administration of hydrocortisone to normal developing mice increases the brain glucose content and cerebral energy reserve. The present report concerns possible mechanisms of this action. Increases in brain glucose (and glycogen) levels were not due to reduction of cerebral metabolic rate, and the effect of hydrocortisone in facilitating transport of hexose from blood to brain was not impressive. Chronic hydrocortisone treatment induced increases in the activities of brain glycerophosphate dehydrogenase and pyruvate carboxylase in vivo; there was no effect on eleven other enzymes of brain glucose and glycogen metabolism. In *normal nursing* mice, hydrocortisone produced consistent elevations in plasma β-hydroxybutyrate (and glycerol) levels. Brain β-hydroxybutyrate levels were also increased. Therefore, the brain glucose concentration may be elevated in these animals because of the availability of an increased supply of ketone bodies as alternative substrates for cerebral oxidative metabolism and biosynthesis. Ketonemia, elevated cerebral glucose and β-hydroxybutyrate concentrations, and increased glycerophosphate dehydrogenase activity in brain suggest possible explanations for the beneficial action of adrenocorticotropic hormone and glucocorticoids in the treatment of infantile myoclonic epilepsy and other neurological disorders.

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Adrenocorticotropic hormone (ACTH) and hydrocortisone are effective in the treatment of infantile myoclonic seizures, but the mechanism of this action is unknown. Stimulated by this enigma, we began a study of the effects of chronic hydrocortisone administration on brain carbohydrate and energy metabolism in young nursing mice. It was known that hydrocortisone can increase total brain content of carbohydrate or glycogen [39]. Using modern techniques of quick freezing and preparation of tissues to preserve glucose and labile high-energy phosphates, we were able to show that hydrocortisone also increases brain glucose concentration and reserves of adenosine triphosphate (ATP) and phosphocreatine [37].

Several possible explanations for the effects of chronic hydrocortisone treatment on brain carbohydrate and energy metabolism were considered: reduction of the cerebral metabolic rate; facilitation of glucose transport from blood into brain; stimulation of the enzymes of gluconeogenesis and glycogenesis in brain; inhibition of the enzymes of cerebral glycolysis and glycogenolysis or of the pentose phosphate pathway; and finally, sparing of glucose utilization in brain by an increased supply of other metabolites as fuels for energy production and substrates for biosynthetic processes. The present study tested these explanations in an effort to gain greater understanding of possible cellular mechanisms underlying the beneficial action of ACTH and glucocorticoids in infantile myoclonic seizures and in other diseases of the central nervous system.

Methods

At birth, litters of Swiss Webster mice were reduced to 12 animals. Starting at 10 days of age, half of each litter were injected subcutaneously twice daily with hydrocortisone sodium succinate, 50 mg per kilogram of body weight dissolved in 20 ml/kg of deionized water. The rest of the litter received an equivalent volume of sterile 0.9% saline solution or were sham injected. To circumvent possible metabolic variations based on circadian rhythms, 1 control and 1 experimental littermate were treated alternately. Animals were killed or other experimental procedures initiated two hours after the last injection (tenth day of treatment). With

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one exception (to be described), animals were killed by decapitation. The head was allowed to drop directly into liquid nitrogen with rapid stirring to facilitate quick freezing. Blood was collected from the severed neck vessels in heparinized capillary tubes (Caraway). Frozen brains were stored at −80°C until the time of dissection.

For determination of cerebral metabolic rate [24], animals killed at zero time were frozen whole. Other animals were decapitated and the severed head was kept at 24°C for 5, 10, 15, or 30 seconds prior to quick freezing in liquid nitrogen. To determine the effect of hydrocortisone treatment on glucose transport from blood into brain, mice were injected with 3-O-[^{14}C]methyl-D-glucose (specific activity, 4.8 mCi/mmol) at a dose of 0.25 μCi per gram intraperitoneally and were decapitated into liquid nitrogen 5 to 20 minutes after injection of the labeled hexose.

Blood capillary tubes were centrifuged promptly at 4°C. Plasma was deproteinized with 10 to 20 volumes of 0.5 M perchloric acid and neutralized with 2 M potassium bicarbonate. Frozen brains (excluding the cerebellum) were dissected in a cryostat at −35°C. Extracts of brain tissue were prepared in perchloric acid [23]. Frozen brain tissue was homogenized by hand in Tenbroeck tissue grinders at 4°C in 15 to 20 volumes of 0.32 M sucrose. Homogenates were frozen immediately at −80°C. Freshly thawed samples were used for measuring the activities of the cerebral "gluconeogenic" enzymes.

Metabolite and Enzyme Assays

With few exceptions, the methods of metabolite assay were those of Lowry and Passonneau [23]. Glycerol was measured according to Burch et al [4], β-hydroxybutyrate by a fluorometric adaptation of the method of Williamson et al [42], and glycogen by the method of Passonneau and Lauderdale [29].

Pyruvate carboxylase and phosphoenolpyruvate carboxykinase enzyme activities were measured by modifications of the techniques of Walter and Stucki [40] and Burch et al [5], respectively. Fructose-1,6-diphosphatase was assayed by a fluorometric adaptation of the method of Krebs and Woodford [20]. Glucose-6-phosphatase was measured in a spectrophotometer as described by Rovainen et al [33].

Glycogen synthetase was measured by the method of Hornbrook et al [16] and glycogen phosphorylase according to Lowry et al [26]. Hexokinase, phosphofructokinase, aldolase, glycerophosphate dehydrogenase, pyruvate kinase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were measured by modifications of the methods of Lowry and his associates [22, 25].

Calculations

The cytoplasmic (c) redox state was calculated from the lactate dehydrogenase (LDH) reaction using the equilibrium constant (K_{LDH}) of 1.11 × 10^{-4} at pH 7.0 [19]:

$$\frac{[NAD^+]_c}{[NADH_2]_c} = \frac{[\text{pyruvate}]}{K_{LDH}[\text{lactate}]}$$

where $[NAD^+]$ = nicotinamide-adenine dinucleotide and $[NADH_2]$ = reduced nicotinamide-adenine dinucleotide. The level of oxaloacetate was calculated from the malate dehydrogenase (MDH) reaction using the equilibrium constant (K_{MDH}) of 2.78 × 10^{-5} at pH 7.0 [19]:

$$\text{Oxaloacetate} = K_{MDH}\,[\text{malate}] \times \frac{[NAD^+]_c}{[NADH_2]_c}$$

and the aspartate aminotransferase (ASP-AT) reaction using an equilibrium constant ($K_{ASP\text{-}AT}$) of 6.61 at pH 7.0 [19]:

$$\text{Oxaloacetate} = \frac{[\alpha\text{-ketoglutarate}]\,[\text{aspartate}]}{K_{ASP\text{-}AT}\,[\text{glutamate}]}$$

Brain pH was evaluated from the creatine phosphokinase (CPK) equilibrium [36]. Hydrogen ion concentration is directly proportional to the mass action ratio, as follows:

$$K_{CPK} = \frac{[\text{creatine}]\,[ATP]}{[\text{phosphocreatine}]\,[ADP]} \times \frac{1}{[H^+]}$$

where ADP = adenosine diphosphate. The cerebral energy charge potential (ECP)—charge of the phosphorylated adenine nucleotide pool—was derived from the formula of Atkinson [3]:

$$ECP = \frac{ATP + 0.5\ ADP}{ATP + ADP + AMP}$$

where AMP = adenosine monophosphate. The cerebral energy reserve (~P)—actual and potential molar equivalents of high-energy phosphate—was calculated by the formula of Lowry et al [24]:

$$\sim P = \text{phosphocreatine} + 2\,(ATP + \text{glucose}) + 2.9\,(\text{glycogen})$$

Cerebral metabolic rate was determined by the closed-system technique of Lowry et al [24]. In this procedure, cerebral metabolic rate—~P use rate—is calculated from the rate of fall of the concentrations of phosphocreatine, ATP, glucose, and glycogen during the first few seconds following decapitation.

Transport of 3-O-[^{14}C]methyl-D-glucose from blood into brain was estimated from the rise in brain/plasma ratios of radioactive carbon 14 (dpm in brain tissue/dpm in plasma) compared with control animals.

The statistical significance of the differences between the means of control and experimental values was determined by Student's t test.

Results

The effect of hydrocortisone on weight gain was not consistent. In many litters no differences were seen; in some the weight gain was less, in others it was greater than in controls. After ten days of hydrocortisone administration the mean body weight, 8.8 ± 0.6 gm (SEM)(N = 52), was 18% lower than in controls (10.8 ± 0.5 gm; N = 53); this difference was significant (p = 0.012). Nevertheless, the range of values was similar (6.9 to 14.6 gm in hydrocortisone-treated mice, 8.1 to 14.0 gm in controls).

Table 1. Effect of Chronic Hydrocortisone Administration on Levels of Selected Metabolites in Mouse Plasma

Metabolite	Plasma Concentration (mM, mean ± SEM) Control Mice	Hydrocortisone-treated Mice	Significance
Glucose	9.28 ± 0.43 (N = 20)	8.15 ± 0.37 (N = 20)	$p = 0.050$
Glycerol	0.186 ± 0.006 (N = 11)	0.355 ± 0.044 (N = 12)	$p < 0.001$
β-Hydroxybutyrate	0.379 ± 0.044 (N = 33)	0.705 ± 0.082 (N = 36)	$p = 0.001$

Table 2. Effect of Chronic Hydrocortisone Administration on Levels of Selected Metabolites in Mouse Brain

Metabolite	Brain Concentration (mmol/kg wet, mean ± SEM) Control Mice (N = 13)	Hydrocortisone-treated Mice (N = 13)	Significance
Glucose	0.98 ± 0.06	1.45 ± 0.08	$p < 0.001$
Glycogen	2.13 ± 0.06	2.48 ± 0.08	$p = 0.004$
G-6-P	0.075 ± 0.002	0.089 ± 0.002	$p < 0.001$
F-6-P	0.0156 ± 0.0005	0.0183 ± 0.0004	$p < 0.001$
F-1,6-P_2	0.121 ± 0.005	0.121 ± 0.004	NS
DHAP	0.055 ± 0.002	0.054 ± 0.001	NS
α-GOP	0.157 ± 0.011	0.148 ± 0.009	NS
P-pyruvate	0.0107 ± 0.0012 (N = 5)	0.0110 ± 0.0007 (N = 6)	NS
Pyruvate	0.139 ± 0.009	0.144 ± 0.008	NS
Lactate	1.78 ± 0.12	1.91 ± 0.10	NS
β-OHB	0.0499 ± 0.0049 (N = 11)	0.0856 ± 0.0076 (N = 11)	$p < 0.001$
Citrate	0.283 ± 0.009	0.311 ± 0.007	$p = 0.024$
α-KG	0.058 ± 0.004	0.077 ± 0.003	$p = 0.003$
Malate	0.482 ± 0.026	0.569 ± 0.030	$p = 0.049$
OAA_{MDH}	0.0061 ± 0.0004	0.0076 ± 0.0004	$p = 0.026$
$OAA_{ASP\text{-}AT}$	0.0028 ± 0.0002	0.0033 ± 0.0002	NS
6-PG	0.0070 ± 0.0004	0.0069 ± 0.0003	NS
Alanine	0.355 ± 0.016	0.404 ± 0.016	NS
Aspartate	3.64 ± 0.18 (N = 6)	3.59 ± 0.15 (N = 6)	NS
Glutamate	10.06 ± 0.13	10.55 ± 0.22	NS
P-Cr	3.11 ± 0.08	3.10 ± 0.06	NS
ATP	2.92 ± 0.08	2.92 ± 0.08	NS
ADP	0.550 ± 0.008	0.544 ± 0.008	NS
AMP	0.058 ± 0.005	0.054 ± 0.004	NS

G-6-P = glucose-6-phosphate; F-6-P = fructose-6-phosphate; F-1,6-P_2 = fructose-1,6-diphosphate; DHAP = dihydroxyacetone phosphate; α-GOP = α-glycerophosphate; P-pyruvate = phosphoenolpyruvate; β-OHB = β-hydroxybutyrate; α-KG = α-ketoglutarate; OAA_{MDH} = oxaloacetate calculated from the malate dehydrogenase equilibrium; $OAA_{ASP\text{-}AT}$ = oxaloacetate calculated from the aspartate-aminotransferase equilibrium; 6-PG = 6-phosphogluconate; P-Cr = phosphocreatine; ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; NS = not significant.

The steroid had no observed effect on the behavior of the animals.

Effect of Hydrocortisone on Selected Metabolite Levels

The plasma of experimental animals was strikingly more lipemic than that of controls. As in our original study [37], plasma glucose levels were slightly lower (12%) in the hydrocortisone-treated mice (Table 1). Concomitantly, plasma glycerol levels increased 91% and β-hydroxybutyrate, 86%.

Chronic hydrocortisone treatment produced a 48% increase in the brain glucose content (Table 2). (In other litters brain glucose concentration doubled [37].) Brain β-hydroxybutyrate levels increased 72%. Statistically significant increases were measured in brain glycogen (16%), glucose-6-phosphate (12%), and fructose-6-phosphate (17%) concentrations. There were no differences in the levels of six other glycolytic intermediates. Hydrocortisone increased the brain citrate content 10%, α-

Table 3. Effect of Chronic Hydrocortisone Administration on Cerebral Metabolite Concentration Ratios and the Cerebral Energy State[a]

Measurement	Control Mice (N = 13)	Hydrocortisone-treated Mice (N = 13)	Significance
Brain/plasma glucose (L/kg)	0.135 ± 0.010	0.192 ± 0.010	$p < 0.001$
Brain/plasma β-OHB (L/kg)	0.114 ± 0.008 (N = 11)	0.099 ± 0.005 (N = 11)	NS
F-1,6-P_2/G-6-P	1.61 ± 0.06	1.38 ± 0.07	$p = 0.021$
F-1,6-P_2/F-6-P	7.90 ± 0.49	6.79 ± 0.31	NS
Lactate/pyruvate	16.2 ± 0.8	15.3 ± 0.9	NS
$[NAD^+]_c/[NADH_2]_c$	576 ± 30	634 ± 46	NS
ATP/ADP	5.18 ± 0.19	5.28 ± 0.18	NS
ECP	0.903 ± 0.004	0.905 ± 0.004	NS
~P (mmol/kg)	17.08 ± 0.34	18.89 ± 0.33	$p = 0.001$
$\frac{[Cr][ATP]}{[P\text{-}Cr][ADP]}$	13.22 ± 0.41	12.47 ± 0.33	NS

[a]All values are given as mean ± SEM.

$[NAD^+]_c$ = cytoplasmic nicotinamide-adenine dinucleotide; $[NADH_2]_c$ = cytoplasmic nicotinamide-adenine dinucleotide, reduced; ECP = energy charge potential; ~P = cerebral energy reserve; Cr = creatine; other abbreviations same as for Table 2.

ketoglutarate 33%, and malate 18%. The results for oxaloacetate were equivocal. There was no change in the levels of 6-phosphogluconate or of the selected amino acids (see Table 2). Concentrations of phosphocreatine, ATP, ADP, and AMP were almost identical to values seen in control animals.

The brain/plasma glucose concentration ratio increased 42% (Table 3). The fructose-1,6-diphosphate/glucose-6-phosphate concentration ratio decreased 19%. The brain/plasma β-hydroxybutyrate ratio, fructose-1,6-diphosphate/fructose-6-phosphate ratio, lactate/pyruvate ratio, and cytoplasmic oxidation-reduction potential were unchanged. There were no differences in the ATP/ADP concentration ratio or in the calculated cerebral energy charge. The cerebral energy reserve increased 11% in experimental animals. Hydrocortisone had no effect on the CPK mass action ratio.

Effect of Hydrocortisone on Cerebral Metabolic Rate

In the face of normal (or reduced) plasma glucose levels, a well-known cause of elevated brain glucose content is reduction in the cerebral metabolic rate. Although the metabolite concentration profile in the brains of hydrocortisone-treated mice (see Tables 2, 3) did not support such an explanation, to rule out this possibility definitively we determined the effect of hydrocortisone treatment on cerebral metabolic rate (see Methods).

The zero-time cerebral metabolite levels shown in Figure 1 are those used for Table 2. In hydrocortisone-treated mice, glucose and glycogen levels remained higher throughout most or all of the ischemic interval. The increased initial reserves of glucose and glycogen available for anaerobic energy production in the experimental animals appear to

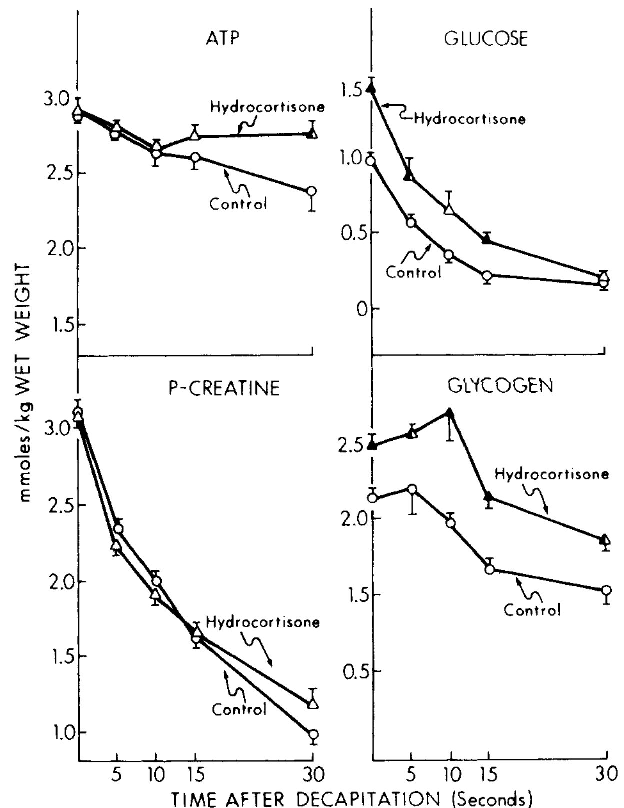

Fig 1. Effect of hydrocortisone on the use of energy-yielding metabolites in mouse brain following decapitation. Each point represents the mean value from 5 to 13 animals. Vertical lines represent ± SEM (not shown if less than width of symbol). Filled symbols indicate a significant difference between control and hydrocortisone values at $p \leq 0.004$, *half-filled symbols at* $p \leq 0.04$. (ATP = *adenosine triphosphate;* P-creatine = *phosphocreatine.)*

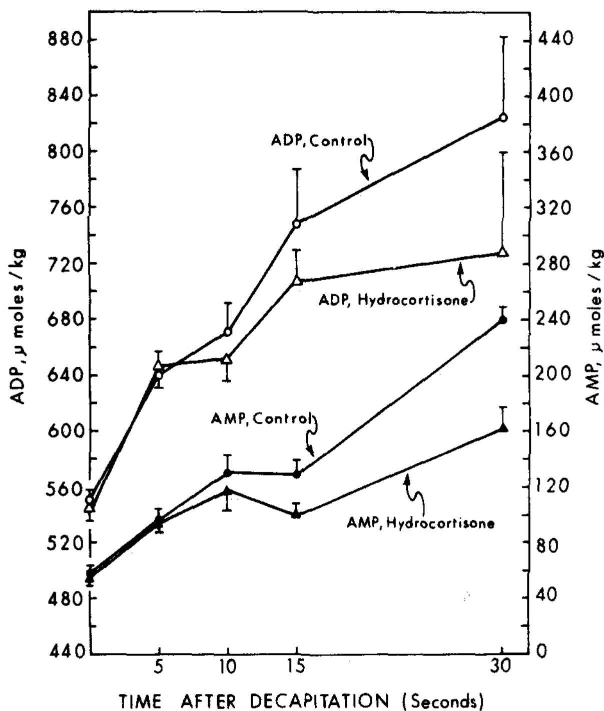

Fig 2. Effect of hydrocortisone on adenosine diphosphate (ADP) *and adenosine monophosphate* (AMP) *levels in brain following decapitation. Animals are those used in Figure 1. The 15-second AMP value in hydrocortisone-treated animals is significantly lower than control at* $p = 0.021$*; the 30-second value differs from control at* $p = 0.013$.

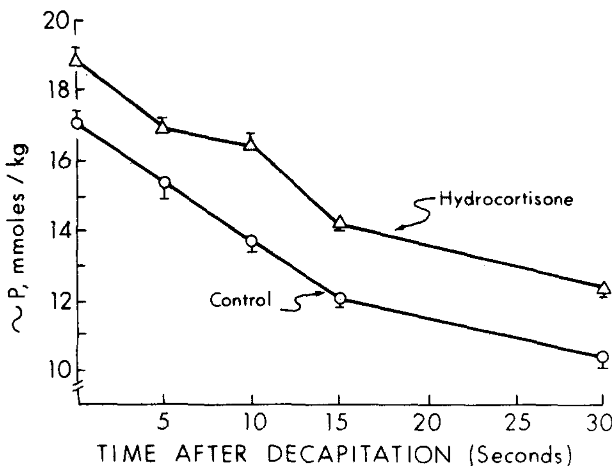

Fig 3. Effect of hydrocortisone on the rate of high-energy phosphate (~P) *use in brain following decapitation. Values are from animals used for Figures 1 and 2. The zero-time and 10-, 15-, and 30-second values are different from control at* $p \leq 0.002$. *The 5-second value differs from control at* $p = 0.012$.

have maintained ATP at higher levels during progressive ischemia. Thirty seconds after decapitation, ATP levels in hydrocortisone-treated animals were not different from the zero-time values; by contrast, ATP levels in control animals had fallen 18% ($p = 0.012$). A trend toward smaller increases of ADP and AMP in the experimental animals reflects this finding

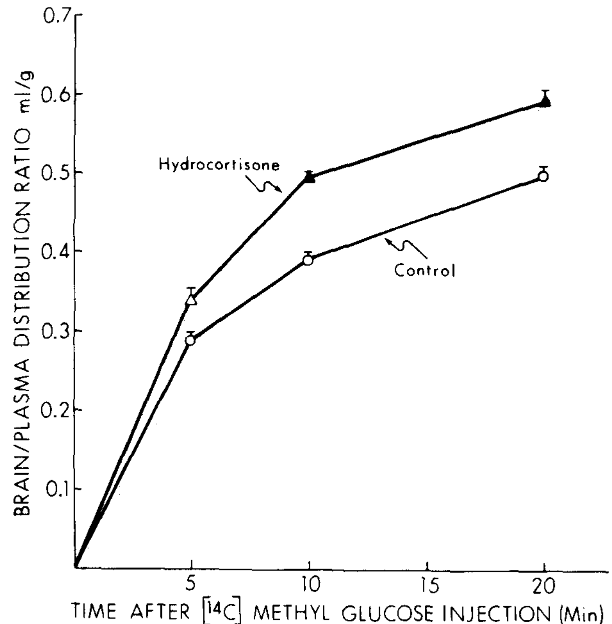

Fig 4. Effect of hydrocortisone on transport of 3-O-[^{14}C]methyl-D-glucose into brain. Each point is the mean brain/blood ^{14}C ratio for 4 to 5 animals. Vertical lines represent ± SEM. The 10-minute ratio is significantly different from control at $p < 0.001$*; the 20-minute ratio differs significantly at* $p = 0.003$.

(Fig 2). AMP values at 15 and 30 seconds of ischemia were, respectively, 30% and 24% lower than in controls (Fig 2).

In both groups of mice, lactate production (not shown) was a close reflection of glucose and glycogen utilization.

In terms of ~P utilization (first 5 seconds of ischemia), the cerebral metabolic rate was calculated to be 20 mmol/kg/min in controls; in the hydrocortisone-treated mice the rate was slightly higher, not lower: 23 mmol/kg/min (Fig. 3). At all ischemic intervals the brain energy content (~P) was significantly higher in the steroid-treated animals (Fig 3).

Effect of Hydrocortisone on Brain Glucose Transport

With normal cerebral metabolic rate, the increase in brain/plasma glucose concentration ratio in hydrocortisone-treated mice (see Table 3) was compatible with facilitation of glucose transport from blood to brain. Fishman and Reiner [13] had found no effect of hydrocortisone on brain glucose transport in developing and adult rats. Nevertheless, we thought it seemed worthwhile to examine the effects of hydrocortisone treatment on brain hexose transport in another species.

In hydrocortisone-treated mice, the brain/plasma distribution ratio of 3-O-[^{14}C]methyl-D-glucose was significantly increased 10 minutes (26%) and 20 minutes (19%) after injection of the isotope (Fig 4).

Table 4. Effect of Chronic Hydrocortisone Administration on Enzyme Activities in Mouse Brain

Enzyme	Brain Activity (μmol/gm/min, mean ± SEM) Control Mice (N = 7)	Hydrocortisone-treated Mice (N = 7)
ENZYMES OF "GLUCONEOGENESIS" (37°C)		
Pyruvate carboxylase	0.589 ± 0.022 (N = 12)	0.662 ± 0.018 (N = 12)[a]
Phosphoenolpyruvate carboxykinase	0.624 ± 0.019	0.574 ± 0.029
Fructose-1,6-diphosphatase[b]	0.150 ± 0.006	0.134 ± 0.004
Glucose-6-phosphatase	0.223 ± 0.020	0.222 ± 0.025
ENZYMES OF GLYCOGEN SYNTHESIS AND GLYCOGEN BREAKDOWN (37°C)		
Glycogen synthetase		
Total	0.160 ± 0.009	0.157 ± 0.010
Active (I) form	0.040 ± 0.003	0.037 ± 0.006
Phosphorylase[b]		
In the presence of 1 mM AMP	1.69 ± 0.16	1.53 ± 0.07
In the absence of 1 mM AMP	0.88 ± 0.11	0.73 ± 0.04
ENZYMES OF THE PENTOSE PHOSPHATE PATHWAY AND GLYCOLYSIS (23°C)		
Glucose-6-phosphate dehydrogenase	0.91 ± 0.04	0.89 ± 0.03
6-phosphogluconate dehydrogenase	0.62 ± 0.01	0.59 ± 0.01
Hexokinase	9.70 ± 0.007	9.72 ± 0.16
Phosphofructokinase	4.61 ± 0.39	5.10 ± 0.38
Fructose-1,6-diphosphate aldolase	6.69 ± 0.08	6.83 ± 0.10
Glycerophosphate dehydrogenase	0.87 ± 0.03 (N = 12)	1.08 ± 0.03 (N = 12)[c]
Pyruvate kinase	21.2 ± 0.6	21.8 ± 0.6

[a]Significance: p vs control, 0.017.
[b]Measured at 23°C.
[c]Significance: p vs control, < 0.001.

However, the consistently lower plasma glucose concentration in the steroid-treated mice would reduce competition for carrier sites, and the increased brain glucose levels would decrease the efflux of labeled methyl glucose from brain. Assuming a Michaelis-Menten constant of 7 mM for glucose transport into brain [6], it was calculated that reduced competition may have enhanced methyl glucose influx by 12% (Regen DM: personal communication, 1976). Conceivably, inhibition of efflux by the higher cerebral glucose content in these animals could account for another portion of the enhancement.

Effect of Hydrocortisone on Selected Enzymes of Carbohydrate Metabolism in Brain

In liver, activities of gluconeogenic enzymes are increased by glucocorticoids [41]. Therefore, the possibility was investigated that the observed increases in cerebral glucose and glycogen levels after hydrocortisone administration might be due to induction of these enzymes in brain.

Clearly, the brain is not a "gluconeogenic" organ. The activities of the potential gluconeogenic enzymes in brain—pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and glucose-6-phosphatase—were 2 to 3% of the activities of the selected glycolytic enzymes (Table 4). Of the four putative gluconeogenic enzymes in brain, only pyruvate carboxylase showed increased activity (12%) from chronic hydrocortisone administration.

Augmented deposition of glycogen in the brains of steroid-treated animals was not associated with increases in glycogen synthetase or decreases in glycogen phosphorylase activities (see Table 4).

Effects of hydrocortisone on the activities of the first two enzymes of the pentose phosphate pathway and of five enzymes in the glycolytic pathway in brain were also examined. Only brain glycerophosphate dehydrogenase activity was affected by hydrocortisone treatment; its activity increased 25%.

Discussion

There are reports of severe growth failure and high mortality following administration of large doses of steroids (equivalent to 150 to 250 mg/kg of hydrocortisone) to very young animals (2 to 6 days old) [14, 17]. We have not seen such an effect in mice treated with hydrocortisone from 10 to 20 days of age at a dosage of 50 mg/kg twice daily; nor were

there any deaths. It may be that previously reported adverse effects of hydrocortisone on body weight reflect the younger age of the animals at onset of therapy, the larger doses of steroids used, or both factors. Findings in one of the cited studies supports this impression: when the total dose of steroid was reduced, "body growth rate recovered and when fully grown the mice reached a size only slightly below that of fed controls" [17].

For several reasons we do not believe that the observed metabolite changes in plasma and brain are related to reduced body weight (? undernutrition). The steroid-induced increase of hepatic glycogen content in these animals [37] does not suggest starvation. We have studied the effect of undernutrition in neonatal rats. There was no change in brain glucose levels despite a 41% decrease in body weight [38]. Finally, brain glucose levels increased in litters of mice in which weight gain was not reduced by hydrocortisone [37].

Metabolite Levels after Hydrocortisone

We have attributed the slight reduction of plasma (and liver [37]) glucose concentrations after chronic hydrocortisone administration in *nursing* mice to increased insulin secretion in response to greater glucose production by glucocorticoid action. There is some support for this explanation. In normal subjects and in patients with mild diabetes, glucocorticoids increased the insulinogenic response to glucose administration despite a deterioration in carbohydrate tolerance; with chronic corticoid administration, glucose tolerance returned to normal [31]. Other explanations are possible. Ketone bodies per se can stimulate the release of insulin [27] (increases in plasma β-hydroxybutyrate levels were consistently seen after chronic hydrocortisone administration). In adult rats, chronic ketosis induced by high-fat feeding was also associated with lowered plasma glucose levels [11].

In *fasting* (intact) animals and in humans, ACTH and glucocorticoids suppressed starvation-induced ketosis or had no effect on levels of circulating free fatty acids, total ketone bodies, or glycerol [28]. In contrast to the negative effects of steroids on plasma ketone body and glycerol levels in the fasted state, chronic hydrocortisone administration to nursing mice doubled plasma glycerol and β-hydroxybutyrate levels. Although other explanations are possible (decreased peripheral utilization or reduced renal excretion of ketones [28]), the findings are compatible with increased lipolysis in adipose tissue and heightened synthesis of ketone bodies by the liver.

None of the studies presented here—measurements of cerebral metabolic rate, brain glucose transport, or brain enzyme activities—offered a satisfactory explanation for the steroid-induced increases in brain glucose or glycogen content. Yet there may be a clue to this problem. The effect of chronic hydrocortisone treatment to increase plasma and brain β-hydroxybutyrate levels was highly consistent.

It is the consensus that brain ketone body utilization is directly proportional to plasma concentration. In developing rats, plasma ketone bodies are ordinarily elevated due to the high fat content of maternal milk, and are an important source of metabolic fuel in brain [15]. In addition, during the crucial period of brain myelination, ketone bodies have been shown to be preferred to glucose for sterol and fatty acid biosynthesis [10, 12, 30]. If they are available in plasma, ketone bodies can also partially replace glucose in brain oxidative metabolism in infants and children [18, 32]. In view of this evidence, we now propose, as originally suggested by Dr David Regen, that the increase in brain glucose (and glycogen) content in young hydrocortisone-treated animals may be due to sparing of glucose oxidation in brain by the increased availability of ketone bodies.

With the exception of the elevated brain glucose and hexose monophosphate content, there was little evidence to support a decrease in cerebral glucose utilization in hydrocortisone-treated mice. Fructose-1,6-diphosphate levels were equal to control values, and lactate was not decreased. Elevated levels of glucose-6-phosphate and fructose-6-phosphate may reflect increased hexokinase and phosphoglucoisomerase activities secondary to a rise in the intracellular glucose concentration rather than inhibition of phosphofructokinase [24].

In adult humans and rats, fasting-induced ketosis is associated with a substantial release of lactate, pyruvate, or both from the brain to venous blood [15, 34]; in rats, infusion of β-hydroxybutyrate caused a further increase in the arteriovenous difference for lactate [34]. The findings suggest inhibition of pyruvate oxidation in brain in ketotic states. Normal levels of lactate and pyruvate in the brains of ketotic hydrocortisone-treated mice do not necessarily negate inhibition of cerebral pyruvate oxidation. Increased efflux of lactate and pyruvate from brain could maintain normal cerebral levels of these metabolites. Evidence that β-hydroxybutyrate transport into brain in vivo appears to be tightly coupled with the countertransport of lactate (or pyruvate or both) supports this explanation [7]. Diversion of pyruvate into the Krebs citric acid cycle by increased pyruvate carboxylase activity is another possibility.

In contrast to the normal levels of glycolytic intermediates at and below the phosphofructokinase step, there were significant increases in levels of selected Krebs citric acid cycle intermediates in the brains of experimental mice. Elevated levels of citrate may reflect augmented synthesis of acetyl coenzyme A (CoA) from oxidation of β-hydroxybutyrate (and

acetoacetate). Brain citrate or acetyl CoA levels were increased in starved rats and in rats with diabetic ketosis [34]. On the other hand, although citrate levels rose slightly in the brains of ketotic rats fed a high-fat diet, there was no change in acetyl CoA levels [11]. Increased carbon dioxide fixation by the augmented activity of brain pyruvate carboxylase [35] could help explain elevated malate and citrate levels in brain.

Influence of Hydrocortisone on Brain Glycerophosphate Dehydrogenase and Pyruvate Carboxylase Enzyme Activities

Of the thirteen selected enzymes related to brain carbohydrate metabolism, only glycerophosphate dehydrogenase and pyruvate carboxylase showed increased activity after chronic hydrocortisone treatment. The developmental pattern of enzyme activities in brain is variable: some rise to high adult values, others decrease to low adult levels. In the latter instance, heightened enzyme activity could reflect delayed cerebral maturation. During development, glycerophosphate dehydrogenase in rat brain increases sharply after 10 days of age; the activity doubles between 21 and 33 days to reach adult levels [21]. If the timing in mice is similar to that in rats, delayed maturation would not explain the increased glycerophosphate dehydrogenase activity in the brains of 20-day-old steroid-treated mice. The activity of pyruvate carboxylase as a function of age has not been studied.

This is the first evidence that hydrocortisone stimulates brain glycerophosphate dehydrogenase activity in normal *intact* animals [9]. In liver, acute or chronic hydrocortisone administration induces the activities of all four regulatory enzymes of gluconeogenesis [41]. Why, in brain, only the activity of pyruvate carboxylase was increased is not known. The change is certainly small, and by itself the finding does not support active gluconeogenesis in brain as an explanation for the elevated cerebral glucose (and glycogen) levels. However, as mentioned before, increased pyruvate carboxylase activity may explain, at least in part, both the normal levels of pyruvate and the higher levels of Krebs citric acid cycle intermediates in brain.

Possible Mechanisms of Clinical Action of ACTH and Glucocorticoids

Glucocorticoids are widely used in the treatment of cerebral edema. The mechanism of any possible beneficial effect is not clear. The results of our study suggest that the effects of glucocorticoids in augmenting glucose and glycogen reserves in brain (and glycogen stores in liver [37, 41]) may play a role in this action. If edematous brain tissue is hypoxic, as seems possible, an increased supply of glucose would be useful since only glucose (and glycogen) can serve ATP production in the absence of oxygen. Maintenance of normal ATP levels in steroid-treated mice during progressive ischemic anoxia supports this speculation. Adlard and De Souza [1] have reported similar results. Dexamethasone *pretreatment* increased total brain carbohydrate content in neonatal rats, and when the animals were subjected to asphyxia, the fall in brain ATP was significantly less than in littermates asphyxiated without being given steroid. In addition, dexamethasone prevented the asphyxia-induced cerebral edema and increased brain sodium/potassium concentration ratio seen in asphyxiated, non-steroid-treated controls [8]. When dexamethasone was administered *after* asphyxia, the increased brain water content and electrolyte abnormalities returned to normal in two hours; by contrast, no such recovery was seen in asphyxiated, non-steroid-treated controls [8].

The value of a ketogenic diet in control of convulsive seizures in humans is well known. In a study of this mechanism, DeVivo et al [11] found that high-fat feeding increased the brain ATP/ADP ratio in rats. They thought this finding could be related to the elevation of electroconvulsive threshold (increased neuronal stability) that develops in this model [2]. While brain glucose (or glycogen) levels rose in all animals treated with hydrocortisone, increases in brain ATP were less consistent [37]. However, since glucose is a major source of ATP production in brain under aerobic conditions, and the only source during anoxia or ischemia, a similar mechanism—increased neuronal stability—might pertain in the hydrocortisone-treated animals. After ten days of hydrocortisone treatment, plasma β-hydroxybutyrate levels in young mice were near those observed in adult rats after three weeks of high-fat feeding [11]. It is not known whether ACTH or glucocorticoid treatment of infants and children with infantile myoclonic seizures is associated with elevations of plasma ketone body levels. Results of this study suggest a need for such an investigation. If ketonemia is induced by steroid therapy (as it is in young mice), the possibility that this mechanism underlies or contributes to the anticonvulsant action of steroids in infants with myoclonic seizures deserves serious consideration.

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