Effect of Acetate on Hypoglycemic Seizures in Mice

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SUMMARY

In order to determine the effects of acetate on signs and symptoms of hypoglycemic seizures, Swiss Webster albino mice were injected intraperitoneally with solutions of NaCl, $NaHCO_3$, NH_4Cl, Na-acetate, or NH_4-acetate, followed by subcutaneous injection of 7 U of insulin/kg body wt. Administration of Na- or NH_4-acetate delayed and reduced the incidence of hypoglycemic reactions. Reinjection with Na-acetate or repeated injections with NH_4-acetate caused a return to normal behavior patterns for 60 and 75%, respectively, of the affected hypoglycemic experimental animals. Injections of control animals with $NaHCO_3$ or NH_4Cl showed that the results were not due to alkalosis or acidosis. Acetate administration significantly increased plasma acetate and citrate, but not glucose, lactate, β-hydroxybutyrate, or acetoacetate concentrations. The results indicate that intraperitoneal administration of acetate directly acted to prevent signs of hypoglycemia from occurring and reversed its manifestations when they were present. The protective effect of acetate suggests that it may serve as a fuel for the brain. DIABETES 28:1022–1026, November 1979.

For many years it was generally accepted that glucose was the sole fuel for the central nervous system.[1] However, Owen et al.'s observations with fasting human subjects suggested that ketosis could prevent the signs and symptoms of hypoglycemia.[2] Subsequent work with animals showed that ketosis induced by fasting[3] or a high-fat diet[4] prevents insulin-induced hypoglycemic seizures. Finally, it has been demonstrated that the brain is capable of utilizing substantial quantities of β-hydroxybutyrate and acetoacetate.[5] Apparently, these compounds are capable of crossing the blood-brain barrier and are metabolized by brain tissue.[6] Furthermore, lactate is also metabolized by lactic dehydrogenase (LDH) in the central nervous system.[7]

Acetate occurs in mammalian plasma in concentrations of less than 0.5 mM.[8] Ruminants absorb a substantial portion of their caloric intake in the form of acetate, but this compound is not a significant fuel for other animals.[9] However, acetyl-CoA synthase, which converts acetate to acetyl-CoA, is abundantly present in many mammalian tissues, including the brain.[9] Because acetate rapidly diffuses across biologic membranes (probably as free acetic acid), the presence of brain acetyl-CoA synthetase suggests the possibility that acetate can serve as a fuel for the central nervous system. Acetate has been shown to be metabolized by rat and bovine cerebral cortex slices in vitro and by intact sheep and human brain.[9–13]

The present experiments were designed to test the possibility that acetate has a protective effect on the signs of insulin-induced hypoglycemia in mice.

METHODS

ANIMALS AND MATERIALS

Male Swiss Webster albino mice, weighing approximately 25 g, were obtained from Simonsen Laboratories (Gilroy, Calif.) and were provided with Wayne Lab-Blox (Allied Mills, Inc., Chicago, Ill.) and tap water ad libitum. Regular insulin, administered subcutaneously, was obtained from Eli Lilly Company (Indianapolis); sodium acetate, grade I, from Sigma Chemical Co. (St. Louis, Mo.); sodium bicarbonate, ammonium chloride, and ammonium acetate from J. T. Baker Chemical Co. (Phillipsburg, N.J.); and 0.9% (150 mM) sodium chloride solution from Travenol Laboratories, Inc. (Deerfield, Ill.). Solutions containing 150 mM $NaHCO_3$, NH_4Cl and Na and NH_4-acetate were prepared with distilled water. The pH of NH_4Cl and Na and NH_4-acetate solutions was adjusted to 7.4 with NaOH and acetic acid, respectively. All salt solutions were injected intraperitoneally (i.p.).

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DESIGN OF EXPERIMENTS

Four experiments were performed.

Experiment 1: Effects of sodium acetate on signs of hypoglycemia. Experimental (N = 10) and control (N = 10) mice received 7 U insulin/kg body wt. The experimental mice were injected with 1.3 ml of 150 mM sodium acetate 15 min before receiving insulin and 50 and 115 min after insulin injection. The control group was injected with 1.3 ml of 150 mM NaCl at the same times. Both groups of animals were observed for signs of hypoglycemia (described below).

Experiment 2: Effects of alkalosis and acidosis on signs of hypoglycemia. Four groups, each consisting of eight mice, were investigated. Groups A and B received 7 U of insulin/kg body wt, while groups C and D received no insulin. Each group was injected with 1.3 ml of the appropriate salt solution i.p. 15 min before and 150 min after insulin injection. Group A received 150 mM NH_4Cl, group B received 150 mM $NaHCO_3$, group C received 150 mM NH_4Cl, and group D received 150 mM $NaHCO_3$. All animals were observed for signs of hypoglycemia.

Experiment 3: Effects of ammonium acetate on signs of insulin-induced hypoglycemia. Both the control (group A, N = 20) and experimental (group B, N = 20) animals received 7 U insulin/kg body wt. Group A received 1.0 ml of 150 mM NaCl 10 min before and 30, 70, 100, and 150 min after the insulin. Group B was injected with 1.0 ml of 150 mM ammonium acetate at the same times. This group of experiments was performed in a blind fashion: the observer did not know which animals received which solutions.

Experiment 4: Effects of insulin and ammonium acetate on plasma metabolites. Both the experimental (group A, N = 80) and the control (group B, N = 80) groups of animals were injected with 7 U of insulin/kg body wt. Group A also received 1.0 ml of 150 mM ammonium acetate 10 min before and 30 and 70 min after insulin. Group B received 1.0 ml of 150 mM NaCl at the same times. In 20 animals from each group, blood was collected prior to insulin administration and 30, 60, and 90 min after insulin injection, and then the animals were decapitated. The blood was heparinized, chilled, and centrifuged at 8700 × *g* for 1 min in a Beckman Microfuge B, and the plasma was analyzed for acetate and organic acids as described below.

SIGNS OF HYPOGLYCEMIA

In all experiments, the animals were observed for objective signs of hypoglycemia, i.e., lethargy (defined as lack of any response when prodded), tremors, splayed hind legs, tonoclonic seizures, medullary breathing, or death.

ANALYTICAL METHODS

Plasma glucose was determined using the Automatic Clinical Analyzer method (Du Pont Instruments, Wilmington, Del.) based on the glucose oxidase technique.[14] Plasma β-hydroxybutyrate, acetoacetate, lactate, pyruvate, and citrate were determined using NAD-linked assays modified from Bergmeyer.[15] Plasma acetate was determined using vacuum microdistillation and gas liquid chromatography.[16]

STATISTICS

All data are expressed as mean ± SEM. Statistical significance was determined by Student's *t* test.

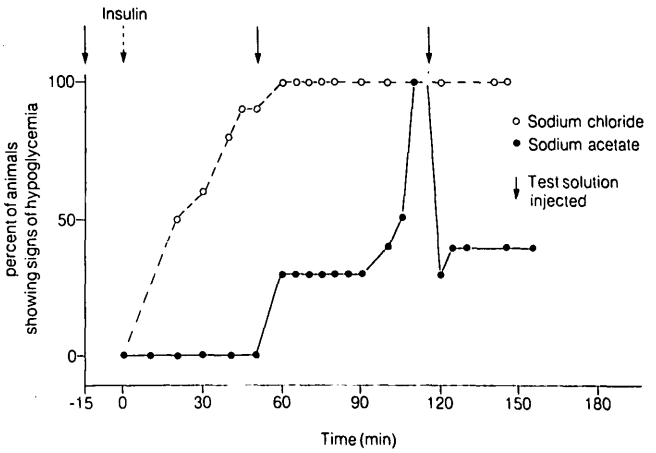


FIGURE 1. Effects of sodium acetate on signs of hypoglycemia (experiment 1). The percent of animals showing signs of hypoglycemia is depicted as a function of time. All animals received 7 U insulin/kg body wt at time = 0. The animals also received either sodium chloride (O) or sodium acetate (●) i.p. at 15, 45, and 120 min.

RESULTS

Effects of sodium on signs of hypoglycemia (experiment 1, Figure 1). Figure 1 shows that 100% of the mice demonstrated signs of hypoglycemia within 1 h after receiving insulin and sodium chloride. In contrast, the animals receiving sodium acetate did not begin to demonstrate manifestations of hypoglycemia until 1 h after insulin injection; by 110 min, all acetate-injected animals also demonstrated signs of hypoglycemia. Although injection of sodium acetate delayed the incidence of hypoglycemia reactions, in some cases the severity of reactions was greater in the acetate-injected group. Three mice in the sodium acetate group died during this period compared with two in the control group.

At 115 min after insulin injection, all mice were reinjected with the appropriate salt solution. Reinjection of NaCl had no detectable effect on the incidence of signs of hypoglycemia. In contrast, 60% of the mice receiving sodium acetate ceased to demonstrate any signs of hypoglycemia and returned to a normal behavior pattern. Thus, intraperitoneal injection of sodium acetate delayed signs of hypoglycemia from occurring and reversed manifestations if they were present.

Effects of alkalosis and acidosis on signs of hypoglycemia (experiment 2, Figure 2). To determine if the effects of sodium acetate administration were related to alkalosis, the effects of sodium bicarbonate and NH_4Cl administration on the signs of insulin-induced hypoglycemia were compared. Figure 2 demonstrates that neither sodium bicarbonate (which produces alkalosis) nor ammonium chloride (which produces acidosis) had an effect on the symptoms of insulin-induced hypoglycemia.

Effects of ammonium acetate on signs of insulin-induced hypoglycemia (experiment 3, Figure 3). Although the results of experiment 1 suggest that sodium acetate could reduce the incidence of signs of hypoglycemia, the increase in mortality rate was attributed to the alkalosis produced by acetate metabolism. For this reason, in experiments designed to determine the effects of repeated acetate administration, ammonium acetate was used instead of sodium acetate. The metabolism of ammonium acetate should

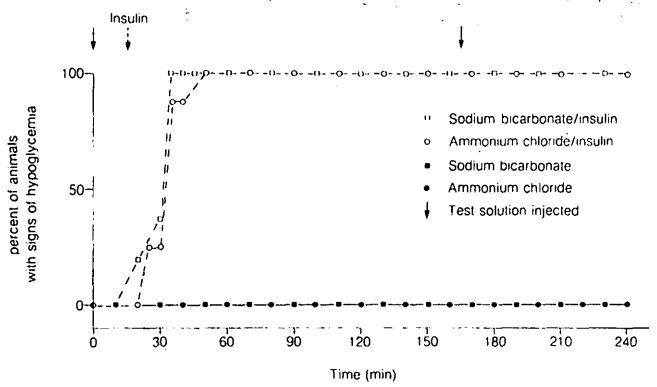


FIGURE 2. Effects of alkalosis and acidosis on signs of hypoglycemia (experiment 2). The data are depicted in a fashion similar to Figure 1. Closed symbols indicate animals that did not receive insulin. Animals depicted by open symbols received insulin at time = 15 min. Sodium bicarbonate or ammonium chloride were injected i.p. at 0 and 165 min.

not affect acid-base status, because both ammonium and acetate ions are rapidly metabolized.

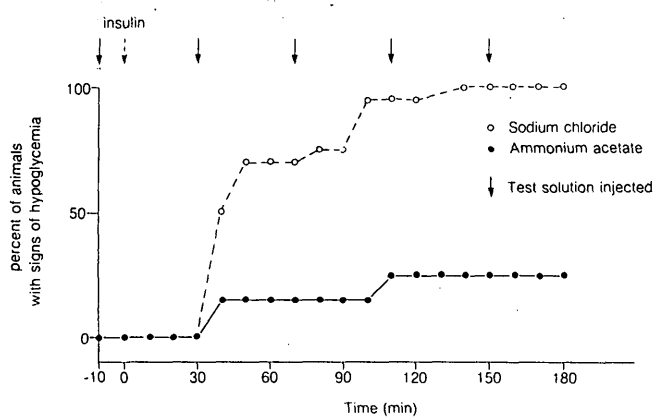
Figure 3 demonstrates that the repeated injections of ammonium acetate reduced the incidence of hypoglycemic seizures to 25% compared with 100% in the control group. The mortality of both groups was the same (2/20).

Effects of insulin and ammonium acetate on plasma metabolites (experiment 4, Figure 4, Table 1). Insulin administration caused a similar decrease of plasma glucose concentrations in both the control and ammonium acetate-injected groups. Intraperitoneal acetate administration caused the plasma acetate concentrations to rise from 0.180 ± 0.025 mM to 2.051 ± 0.214 mM. The administration of acetate prevented the fall of plasma citrate concentrations occurring in the control animals, but had no significant effects on plasma concentrations of other metabolites.

DISCUSSION

The present experiments indicate that intraperitoneal administration of sodium acetate or ammonium acetate can prevent and reverse the signs of insulin-induced hypoglycemia. There are several possible interpretations of these results. One possibility is that the alkalosis caused by acetate metabolism prevented the signs of hypoglycemia. However, alkalosis or alkalemia is known to increase neuromuscular

FIGURE 3. Effects of ammonium acetate on signs of insulin-induced hypoglycemia (experiment 3). Data are depicted in a fashion similar to Figure 1. All animals received insulin at time = 0. Animals received i.p. injections of sodium chloride (○) or ammonium acetate (●) at the times indicated (↓).



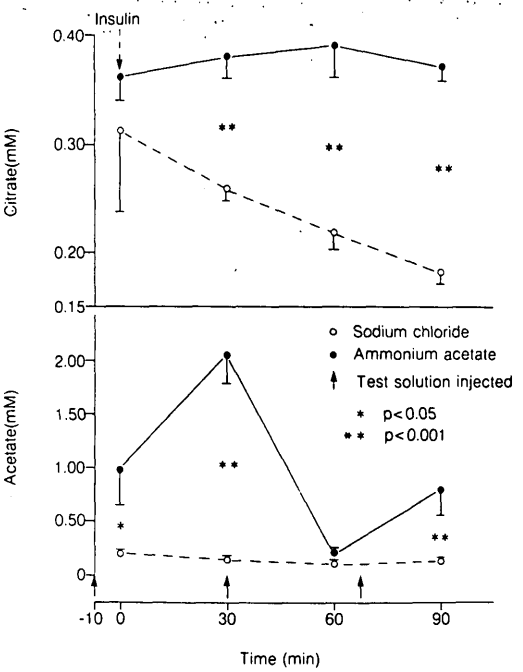


FIGURE 4. Effects of insulin and ammonium acetate on plasma metabolites (experiment 4). Plasma concentrations of citrate (upper panel) and acetate (lower panel) are depicted as a function of time. Insulin administered at time = 0. Intraperitoneal injections of sodium chloride (○) or ammonium acetate (●) were administered at the indicated times (↓). Statistical significance between groups is indicated (*$P < 0.05$, **$P < 0.01$).

irritability and results in tremors, tetany, and seizures.[17] Therefore, the protective effect of acetate was probably not caused by alkalosis. In fact, the high mortality of the experimental mice in the first experiment (Figure 1) may have been due to a potentiating effect of alkalosis on hypoglycemic seizures. Furthermore, the results of the experiments in Figure 2 indicate that neither $NaHCO_3$ nor NH_4Cl affected the incidence or severity of hypoglycemic seizures.

A second possibility is that the administration of acetate produced resistance to the effects of insulin and prevented the development of hypoglycemia. However, the finding that plasma glucose values were not affected by acetate administration discounts this possibility.

Other substrates such as β-hydroxybutyrate, acetoacetate, and lactate can be used as fuel by the brain. Therefore, a third possible explanation for the present results is that the administration of acetate caused production of another metabolite that could then be used by the central nervous system. This possibility seems unlikely because none of the metabolites measured were increased by acetate administration. The concentration of citrate in the experimental animals remained stable, whereas control citrate concentrations diminished (Figure 4 and Table 1); but the high molecular weight and multivalency of citrate make it an unlikely candidate as a substrate for central nervous tissue.

The fourth and most probable explanation is that the administered acetate was utilized by brain tissue as a supplementary fuel when glucose availability was diminished by insulin.

The biologic significance of the present findings remains uncertain because plasma acetate concentrations are too low in most species to serve as a significant source of energy. The role of acetyl-CoA synthetase in mammalian tis-

TABLE 1
Effects of acetate on organic acids

			Time after insulin injection			
			0 min	30 min	60 min	90 min
NaCl (control)	Glucose	(mg/dl)	98 ± 20 (8)	69 ± 4 (10)	77 ± 8 (6)	102 ± 14 (14)
	Acetate	(mM)	0.194 ± 0.025 (9)	0.153 ± 0.021 (10)	0.111 ± 0.013 (10)	0.129 ± 0.021 (13)
	Lactate	(mM)	4.92 ± 0.59 (10)	5.11 ± 0.41 (10)	5.36 ± 0.58 (10)	3.03 ± 0.26 (9)
	Pyruvate	(mM)	0.053 ± 0.008 (10)	0.108 ± 0.013 (10)	0.112 ± 0.010 (10)	0.045 ± 0.00 (9)
	β-OH-Butyrate	(mM)	0.206 ± 0.008 (10)	0.206 ± 0.055 (10)	0.197 ± 0.032 (10)	0.296 ± 0.06 (9)
	Acetoacetate	(mM)	0.045 ± 0.012 (10)	0.041 ± 0.009 (10)	0.022 ± 0.002 (10)	0.022 ± 0.00 (9)
	Citrate	(mM)	0.312 ± 0.075 (10)	0.259 ± 0.014 (10)	0.219 ± 0.016 (10)	0.183 ± 0.00 (9)
NH_4-acetate (experimental)	Glucose	(mg/dl)	136 ± 16 NS (9)	77 ± 5 NS (6)	78 ± 11 NS (5)	107 ± 14 NS (7)
	Acetate	(mM)	0.987 ± 0.330 * (8)	2.051 ± 0.214 † (6)	0.196 ± 0.051 NS (7)	0.794 ± 0.223 † (8)
	Lactate	(mM)	6.87 ± 0.52 * (10)	5.83 ± 0.76 NS (10)	6.18 ± 0.36 NS (10)	5.31 ± 0.46 ‡ (10)
	Pyruvate	(mM)	0.182 ± 0.045 † (9)	0.138 ± 0.008 NS (10)	0.119 ± 0.012 NS (10)	0.112 ± 0.019 † (10)
	β-OH-Butyrate	(mM)	0.313 ± 0.170 NS (10)	0.158 ± 0.077 NS (10)	0.042 ± 0.027 † (10)	0.118 ± 0.06 NS (10)
	Acetoacetate	(mM)	0.061 ± 0.016 NS (9)	0.033 ± 0.004 NS (10)	0.078 ± 0.019 NS (10)	0.034 ± 0.001 * (10)
	Citrate	(mM)	0.361 ± 0.021 NS (10)	0.380 ± 0.021 † (10)	0.390 ± 0.023 † (10)	0.373 ± 0.016 † (10)

Data are expressed as mean ± SEM. The number of animals used to obtain each value is shown in parentheses. Statistical significance was determined by the unpaired Student's *t* test. NS = not significant. *P < 0.05. †P < 0.01. ‡P < 0.001.

sue has not been elucidated. Therefore, the finding that acetate can prevent hypoglycemic seizures has relevance only when large quantities of acetate are exogenously administered or endogenously provided. The major medical use of sodium acetate is in hemodialysis. During hemodialysis, approximately 1 mol of acetate is transferred into the patient, and plasma acetate concentrations may rise to as high as 10 mM.[8] Hypoglycemic reactions occasionally occur if glucose-free dialysate is used. These patients may be protected by elevated plasma acetate concentrations, and the fall in plasma acetate after dialysis might allow the development of hypoglycemic seizures. Plasma acetate concentrations are also high following alcohol ingestion because alcohol is metabolized to acetaldehyde and then to acetate. It is possible that the elevated plasma acetate concentrations associated with alcohol ingestion might initially protect against the signs of alcohol-induced hypoglycemia.[18] When the acetate is subsequently metabolized, the signs and symptoms of alcohol-induced hypoglycemia may then become manifest.

The present findings that acetate prevents and reverses hypoglycemic seizures are not unequivocal proof that acetate is metabolized by the central nervous system. Direct quantitation of acetate uptake by the brain, by measuring arteriovenous acetate concentration differences and cerebral blood flow, has established that acetate is metabolized by the brain in vivo,[15,16] and the previous reports concerning the high permeability of biologic membranes to acetate,[19] the presence of acetyl-CoA synthase in the brain,[9] together with the present results, add to evidence that acetate may serve as a fuel to the brain.

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