Mechanisms of Seizures and Coma in Hypoglycemia

EVIDENCE FOR A DIRECT EFFECT OF INSULIN ON ELECTROLYTE TRANSPORT IN BRAIN

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ABSTRACT The mechanisms involved in the production of hypoglycemic coma were studied in rabbits. Measurements were made in brain, cerebrospinal fluid (CSF), and plasma of osmolality, Na^+, K^+, Cl^-, water content, exogenous insulin, glucose, lactate, and glutamate, while pH, P_{CO_2}, P_{O_2}, and bicarbonate were evaluated in arterial blood. 35 min after i.v. injection of insulin (50 U/kg), plasma glucose did not change, but brain K^+ content increased significantly. Grand mal seizures were observed in unanesthetized animals (±SD) 133±37 min after administration of insulin, at a time when brain glucose was normal, but brain tissue content of Na^+, K^+, osmoles, and water was significantly greater than normal. Coma supervened 212±54 min after insulin injection, at which time brain glucose, lactate, and glutamate were significantly decreased. At both 35 and 146 min after insulin administration, exogenous insulin was present in brain, but not in the CSF. After 208 min of insulin administration, animals were given i.v. glucose and sacrificed 35 min later. Most changes in the brain produced by hypoglycemia were reversed by the administration of glucose. Hypoxia ($P_{O_2} = 23$ mm Hg) was produced and maintained for 35 min in another group of animals. Hypoxia caused brain edema but did not affect brain electrolyte content. However, brain lactate concentration was significantly greater than normal. The data indicate that the seizures noted early in the course of insulin-induced hypoglycemia are temporally related to a rise in brain osmolality secondary to an increased net transport into brain of Na^+ and K^+, probably caused by insulin, per se. As hypoglycemia persists, there is also a depletion of energy-supplying substrates (glucose, lactate, glutamate) in the brain, an event which coincides with the onset of coma. The brain edema observed during hypoxia is largely due to an increase in brain osmolality secondary to accumulation of lactate.

This study was presented in part at the 26th Annual Meeting, Western Society for Clinical Research, Carmel, Calif., 3 February 1973, and at the 26th Annual Meeting, American Academy of Neurology, San Francisco, Calif., 27 April 1974. VA project No. 0508-02.

Dr. Arieff is a Clinical Investigator of the Veterans Administration. Dr. Doerner and Dr. Zelig were recipients of Summer Fellowships for Medical Students.

Received for publication 7 December 1973 and in revised form 20 May 1974.

INTRODUCTION

Hypoglycemia may be associated with tremulousness, cold sweating, headache, and confusion. In addition, a multitude of neurological signs and symptoms, such as delirium, hypothermia, brainstem dysfunction, stroke-like illness, and focal or generalized seizures may occur (1). Such neurological manifestations may progress to coma and death (1). The symptoms of hypoglycemia, as well as autopsy findings, suggest the presence of increased intracranial pressure, and it is possible that cerebral edema may underlie many of the symptoms of hypoglycemic coma. Since in both patients and experimental animals dying with hypoxia, the gross and microscopic findings in the brain are similar to those seen with hypoglycemia, it has been postulated that the pathophysiologic events underlying the symptomatology and coma in these two conditions may be similar (1–4). It has been also suggested that with both hypoglycemia and hypoxia, a decrease in cerebral oxygenation might disrupt cell membrane-bound Na^+-K^+ activated ATPase,

with a net movement of Na^+ into brain (4). This increment in brain Na^+ would raise brain osmolality which in turn would cause movement of water into brain, and hence cerebral edema would ensue.

Other studies have shown that hypoglycemia does not cause a significant fall in brain ATP or phosphocreatine (5, 6). Duffy, Nelson, and Lowry (4), therefore, postulated that hypoglycemic coma may result from a lack of specific energy-supplying substrate, such as glucose and its metabolites. Such metabolites might include lactate and glutamate, which can probably serve as energy sources for the brain under hypoglycemic conditions (2, 7, 8).

Data from our laboratory have suggested that, in the absence of hyperglycemia, insulin may directly enhance transport of Na^+ and K^+ into brain (9). Such an effect of insulin may be present during hypoglycemia, and it may lead to accumulation of these ions in brain, causing cerebral edema.

Although cerebral edema may be responsible for both hypoglycemic and hypoxic coma, the events leading to brain edema are probably complex and may be different in these two pathological entities. The present study was undertaken to investigate the changes in brain water and electrolyte metabolism that occur during hypoglycemia and hypoxia in an effort to define the mechanisms of the coma in these conditions.

METHODS

Studies were performed in five groups of New Zealand white rabbits which were maintained on an ad libitum water intake and Purina chow diet (Ralston Purina Co., St. Louis, Mo.). The animals were fasted for 15 h before experiments. Except for rabbits in group I, all animals were studied under sodium pentobarbital anesthesia. Ventilation was maintained with a Harvard respirator (no. 661, Harvard Apparatus Co., Inc., Millis, Mass.) via a tracheostomy tube. Tidal volume was based on the weight of the animal (10), and minor adjustments were made throughout the experiment to maintain P_{CO_2} around 35 mm Hg at a respiratory rate of 25/min.

In preliminary experiments, it was found that after the intravenous injection of insulin, grand mal seizures were observed after 146 min, and the animals became unresponsive to noxious stimuli after 208 min. In addition, Zierler and Rabinowitz (11) had previously shown that after 35 min, insulin increased transport of K^+ into skeletal muscle without affecting plasma glucose. Thus, we elected to study changes in brain water and solute at 35, 146, and 208 min after insulin administration.

Group I. 10 awake rabbits were given 50 U/kg of insulin (Iletin, Eli Lilly and Co., Indianapolis, Ind.) and were observed for the appearance of seizure activity and coma. Blood samples were obtained before the injection of insulin, at the time when seizures were first observed, and at the appearance of coma (unresponsiveness to noxious stimuli).

Group II. 19 animals were given 50 U/kg of insulin and were sacrificed after 35 min (6 rabbits), 146 min (7 rabbits), and 208 min (6 rabbits).

Group III. Eight rabbits received 50 U/kg of insulin. After 208 min of insulin administration they were given an intravenous injection of either 5 or 13 ml of 2.8 M glucose solution. They were sacrificed 35 min later.

Group IV. 10 rabbits were treated in a similar manner as group II, but the insulin they received was a mixture of regular and ^{125}I-labeled insulin. Regular insulin was mixed with ^{125}I-labeled insulin (Imusay, Abbott Laboratories, North Chicago, Ill., 250 μCi/ml) to attain a concentration of 50 U regular insulin/ml and 1 μCi/2 U insulin. The animals were sacrificed at 35 and 146 min after the administration of insulin mixture. Blood, and cerebrospinal fluid (CSF) [1] samples were obtained at the time of sacrifice, and the simultaneous levels of exogenous insulin were determined in plasma, CSF, and brain.

Group V. Eight animals were made hypoxic by having them breath a mixture of 5% O_2–95% N_2 via a tracheostomy tube. The arterial P_{O_2} was maintained at about 23 mm Hg for 35 min, and anima'ls were then sacrificed.

At the beginning of experiments, 50 μCi of $^{35}SO_4^=$ (New England Nuclear, Boston, Mass., sp act 825 mCi/mmol) was given intravenously, and 10 min before the conclusion of each experiment in groups II, IV, and V, the animals were given 25 μCi of ^{125}I-labeled albumin intravenously (E. R. Squibb & Sons, New York). In animals of group I, only plasma glucose was measured. In all other groups, measurements were made in plasma, CSF, and brain of Na^+, K^+, Cl^-, lactate, glucose, glutamate, osmolality, and exogenous insulin. In addition, the pH, P_{CO_2}, P_{O_2}, and bicarbonate were determined in arterial blood and the [^{125}I]-albumin space, extracellular space (ECS), water content, and inorganic sulfate ($^{35}SO_4^=$) space (relative to plasma) were measured in brain. When ^{35}S-labeled sulfate ($^{35}SO_4^=$) is given intravenously, about 5–10% is incorporated into brain tissue proteins or other macromolecules (12). However, we have previously shown that deproteinization of brain tissue (or plasma) precipitates the bound sulfate, but does not precipitate free sulfate (13). Thus, when brain and plasma have been deproteinized, the ratio of brain [$^{35}SO_4^=$] (cpm/g)/plasma [$^{35}SO_4^=$] (cpm/g) gives a representative value for the distribution of inorganic sulfate in brain relative to plasma.

The skull was opened with a trephine and rongeur, samples of CSF were obtained, and the brain was then removed as previously described (9). The cerebral hemispheres were separated from the rest of the brain and immersed in a Dewar flask filled with liquid nitrogen. The elapsed time from removal of the brain to its immersion in liquid nitrogen was less than 8 s in all cases. The cerebral hemispheres were then pulverized to a powder while under liquid nitrogen. Three samples, each about 0.4 g, were placed in conical glass flasks, weighed, dried in an oven at 100°C for 48 h, and then reweighed in order to determine water content. These samples were then extracted for 24 h in 0.75 N HNO_3, and the supernate was used for the measurement of Na^+, K^+ and Cl^- (14). Three additional 0.4-g samples were extracted in cold 185 mM trichloroacetic acid (TCA) and the supernate used for the determination of lactate, free glucose, glutamate, and $^{35}SO_4^=$ activity (13, 15–17). The methods were modified for use with tissue extracts. These modifications, along with appropriate recovery experiments, have previously been described (9). Two unprocessed 0.2-g tissue samples were used for the determination of ^{125}I activity (13).

[1] *Abbreviations used in this paper:* CSF, cerebrospinal fluid; ECS, extracellular space; TCA, trichloroacetic acid.

Five additional 0.3-g samples were extracted in boiling distilled water and used for the determination of brain tissue osmolality. The previously described method for brain osmolality determination (18) has been further evaluated in normal, uremic, and hemodialyzed dogs (19) and in hyperglycemic rabbits (9). Additionally, a comparison was made of the simultaneously measured osmolalities of brain tissue and CSF in six groups of rabbits subjected to various experimental manipulations, who had been in a steady state for at least 2 h.

The brain ECS was evaluated as the Cl^- space relative to cisternal CSF (20), correcting for the brain intracellular Cl^- content (21). The brain intracellular concentration and content of glucose, Na^+, and K^+ were calculated relative to the concentrations of these substances in cisternal CSF (20) by using the corrected Cl^- space as being representative of the brain ECS (21).

Exogenous (^{125}I) insulin was determined in plasma, CSF, and brain of group IV animals in the following manner. Three preweighed samples of CSF, plasma, brain, and labeled insulin were counted in an automatic gamma counter (Nuclear Chicago Corp., Des Plaines, Ill.) both before and after extraction with 185 mM TCA. The results of all of the above were converted into disintegrations per minute per gram of either plasma, CSF, or brain. The TCA-extractable fraction was assumed to represent insulin metabolites (22) and was subtracted from the activity of the unprocessed samples to obtain the radioactivity which was actually due to insulin. The radioactivity which was extracted by TCA from the labeled insulin was found to be essentially zero. The insulin concentration of CSF, plasma, and brain was calculated by comparison to the ^{125}I activity of the insulin standard (50 U/ml) which had been injected into the animals.

The arterial pH, P_{CO_2}, and P_{O_2} were measured on a Radiometer-Copenhagen BMS 3-PHM 71 blood microsystem acid-base analyzer (Radiometer Co., Copenhagen, Denmark), while the bicarbonate was calculated from the Siggaard-Andersen alignment nomogram (1962). Lactate and glutamate were determined by measuring the conversion of NAD to $NADH_2$ in a Hitachi double beam spectrophotometer (Hitachi Ltd., Tokyo, Japan) at 340 nm (15, 16), and glucose was measured with *o*-toluidine (17).

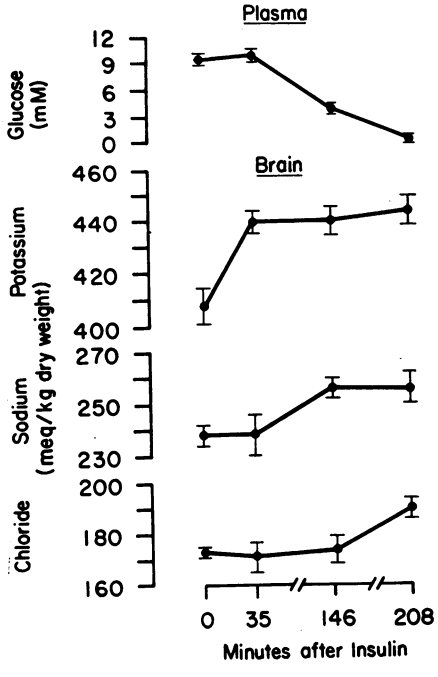

FIGURE 1 The effects of intravenous insulin on plasma glucose and brain tissue electrolyte content. There is a significant increase in brain K^+ after 35 min, with no change in plasma glucose. After 146 min, brain Na^+ increases significantly, while plasma glucose is falling, and after 208 min, brain Cl^- is increased and gross brain edema is present.

RESULTS

The intravenous administration of 50 U/kg insulin to 10 normal awake rabbits produced grand mal seizures within 133±11 (SE) min at a time when plasma levels of glucose had fallen from 5.7±0.3 to 1.7±0.1 mmol/liter ($P < 0.01$). Despite the fact that grand mal convulsions were occurring intermittently, the animals were responsive to loud noise and noxious stimuli between seizures. 212±16 min after the injection of insulin, the animals became unresponsive to noxious stimuli, at which time plasma glucose levels were 1.3±0.1 mmol/liter. 35 min after the injection of ^{125}I-labeled insulin, the plasma level of exogenously administered insulin was 707±102 mU/ml. No exogenous insulin was detected in CSF, and its concentration in brain was 21±8 mU/g wet wt. [^{125}I]Albumin space was 3.0±0.7 g/100 g wet wt; it appears, therefore, that essentially all insulin present in brain was confined to the plasma. After 146 min, the plasma level of exogenous insulin was 533 ±103 mU/ml. Again, no insulin was detected in CSF, and brain insulin was 52±11 mU/g. Since brain [^{125}I]-albumin space was 2.3±0.1 g/100 g wet wt, one can estimate that about 41 mU of insulin/g tissue may have been present in brain. The quantities of insulin which were administered, while large, were necessary in order to produce significant hypoglycemia in the rabbit. Smaller doses of insulin (25 U/kg) did not consistently lower plasma glucose within 4 h. These levels of plasma insulin, however, are considerably larger than those which have been observed in patients with insulinoma, or in those with reactive hypoglycemia who have received leucine infusions (23).

The effects of the intravenous administration of insulin on plasma glucose and the content of K^+, Na^+, and Cl^- in brain tissue are shown in Fig. 1. The effects of the various experimental maneuvers on glucose and electrolyte concentration in plasma and CSF and on brain electrolyte, glucose, lactate glutamate, and water content are presented in Tables I, II, and III. 35 min after injection of insulin, there was no significant change in the plasma level of glucose; the osmolality and the concentrations of Na^+, K^+, Cl^-, and lactate in

TABLE I

Effect of Insulin and Hypoxia on the Composition of Plasma

	Na^+	K^+	Cl^-	Osmolality	Glucose Initial	Glucose Final	pH	P_{CO_2}	P_{O_2}	HCO_3^-	H^+
		meq/liter		*mosmol/kg*	*mmol/liter*			*mm Hg*		*meq/liter*	*nmol/liter*
Normal, $n = 7$	140	3.55	99	301	9.1	—	7.38	37.2	60	22.8	42
	±1	0.13	±3	±2	±0.4	—		±1.7	±4	±0.5	±3
Insulin, *35 min*, $n = 6$	139	2.43*	110*	292‡	10.3	10.0	—	—	—	—	—
	±0.4	±0.18	±1	±2	±0.6	±0.7		—	—	—	—
Insulin, *146 min*, $n = 7$	142	3.21	108‡	292‡	9.0	4.4*	7.34	34.0	71.7	18.2	46
	±1	±0.23	±2	±2	±0.5	±0.4		±2.1	±3.9	±1.4	±2
Insulin, *208 min*, $n = 6$	142	2.78*	104	292‡	11.4	2.0*	7.31	35.5	62.3	18.1	49
	±4	±0.16	±1	±3	±1.8	±0.3		±1.8	±3.4	±0.4	±2
Insulin, *208 min*, +glucose, $n = 10$	134‡	2.69*	108‡	301	2.3*	17.6‡	—	—	—	—	—
	±2	±0.19	±2	±2	±0.4	±3.0		—	—	—	—
Hypoxia, *35 min*, $n = 8$	138	6.93*	102	314‡	—	18.6*	7.17*	16.5*	22.9*	6.3*	62*
	±1	±0.65	±1	±4	—	±2.3		±1.8	±1.4	±0.6	±5

Values are presented as Mean±SE.
* $P < 0.01$ vs. normal.
‡ $P < 0.05$ vs. normal.

both plasma and CSF were also unchanged. However, the brain content of K^+ was significantly greater ($P < 0.001$) than that in normal rabbits (Table III). Virtually all of the increase in brain K^+ was intracellular. Although CSF K^+ did not change (Table II), the brain intracellular K^+ content increased from 407±6 to 437±3 meq/kg intracellular dry wt ($P < 0.01$, Fig. 2). The water content of brain tissue was significantly higher than normal values (Table III, $P < 0.05$) by about 6%, with the increase being of approximately equal distribution between intracellular and extracellular compartments (Table III, Fig. 2). Despite the significant increase in brain K^+ content, the brain intracellular concentration of K^+ (154±1 meq/kg intracellular H_2O) was not different from the normal value (152±1 meq/kg intracellular H_2O) because of the accompanying increase in brain water (Fig. 2). The brain osmolality and contents of glucose, lactate, Na^+, and Cl^- were not different from values seen in normal rabbits, and the brain ECS was not different from normal.

After 146 min of insulin administration, the brain osmolality (322±3 mosmol/kg H_2O) was significantly greater than that of plasma (295±2 mosmol/kg) and that of brain of normal rabbits (300±8 mosmol/kg H_2O), with $P < 0.01$. The brain intracellular K^+ content (437±5 meq/kg intracellular dry wt) was similar to the value observed after 35 min, but brain intracellular Na^+ content was 94±2 meq/kg intracellular dry wt, a value significantly different ($P < 0.01$) from that observed in normal rabbits (75±2 meq/kg intracellular dry wt). Although plasma glucose had fallen significantly (Fig. 1), brain intracellular glucose concentration was not different from the value observed in

TABLE II

Effect of Insulin and Hypoxia on the Composition of CSF

	CSF					
	Na^+	K^+	Cl^-	Osmolality	Glucose	Lactate
		meq/liter		*mosmol/kg*	*mmol/liter*	*mmol/liter*
Normal, $n = 7$	155±2	2.88±0.04	122±1	305±2	4.6±0.4	2.92±0.36
Insulin, *35 min*, $n = 6$	150±1	2.84±0.08	128±2	299±3	4.5±0.4	2.92±0.41
Insulin, *146 min*, $n = 7$	148±2	2.79±0.04	124±7	297*±1	2.7*±0.4	2.93±0.28
Insulin, *208 min*, $n = 6$	147±3	2.81±0.12	124±5	296‡±3	2.7‡±0.7	2.39±0.12
Insulin, *208 min*, +glucose, $n = 10$	150±2	2.88±0.07	133±2	304±2	5.3±0.5	2.85±0.22
Hypoxia, *35 min*, $n = 8$	156±5	3.54*±0.38	126±3	309±8	4.2±0.5	9.98*±1.68

Values are presented as Mean±SE.
* $P < 0.01$ vs. normal.
‡ $P < 0.05$ vs. normal.

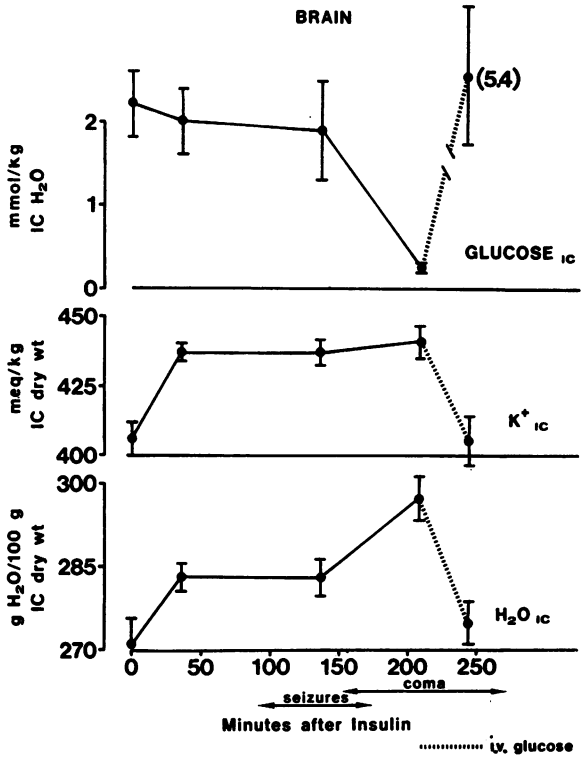

FIGURE 2 Changes in brain intracellular (IC) glucose concentration and in brain intracellular content of water and K^+. 35 min after intravenous insulin, when brain glucose is normal, there is a significant rise in brain intracellular K^+ content. After 133±37 min (±SD), unanesthetized animals manifest grand mal seizures, at which time brain intracellular content of Na^+, K^+, and water are significantly greater than normal, but brain intracellular glucose concentration is normal. After 212±54 min, animals are comatose (unresponsive to noxious stimuli). At this time, when compared to normal values, brain intracellular glucose is significantly reduced, while intracellular water is significantly elevated. When intravenous glucose is given to such hypoglycemic, comatose animals, there is reversal of most of the changes in brain glucose, water, and electrolytes within 35 min.

normal rabbits (Fig. 2). Thus, grand mal seizures, which were observed 133±11 min after insulin administration, occurred while brain glucose was normal. There were no changes in the brain ECS, $^{35}SO_4^=$ space, or [^{125}I]albumin space, and brain concentrations of lactate and glutamate were not different from normal values. There were no significant changes in CSF or plasma concentration of Na^+, K^+, Cl^-, or osmoles, and the arterial P_{O_2}, pH, and bicarbonate were unaltered.

208 min after the injection of insulin, the concentrations of glucose in plasma and CSF fell to 2.0±0.3 and 2.7±0.7 mM, and there were corresponding decrements of osmolality in plasma to 292±3 mosmol/kg and in CSF to 296±3 mosmol/kg. There were no significant changes in the levels of Na^+ and Cl^- in plasma and CSF, but the K^+ concentration in plasma fell significantly. The brain was grossly edematous and bulged through a trephine opening in the skull. There were no significant changes in brain content of Na^+ and K^+ from values observed at 146 min after insulin injection, but Cl^- content increased significantly ($P < 0.01$).

The brain intracellular and extracellular water content were both found to be further increased, to 299±4 and 117±2 g/100 g dry wt, respectively, (Fig. 2), values significantly different ($P < 0.01$) from those observed in normal rabbits (268±5 and 104±2 g/100 g dry wt). The increase in brain water content resulted in a fall of brain osmolality to normal values (Table III). Brain intracellular contents of Na^+ and K^+ were not different from values observed after 146 min (Fig. 2), and their concentrations ($Na^+ = 28.3 \pm 0.6$ and $K^+ = 150 \pm 2$ meq/kg intracellular H_2O) were not different from normal values ($Na^+ = 28.2 \pm 0.5$ and $K^+ = 152 \pm 1$ meq/kg intracellular H_2O). There was a significant increase in the brain ECS (Table III), but not in the $^{35}SO_4^=$ or [^{125}I]albumin spaces.

The concentrations in brain tissue of glucose, of lactate, and of glutamate all were significantly lower than in normal rabbits (Table III). The brain intracellular glucose concentration was 0.25±0.02 mmol/kg intracellular H_2O, a value significantly less ($P < 0.01$) than the value in normal rabbits (2.3±0.4 mmol/kg intracellular H_2O). Thus, coma, which occurred 212±16 min after insulin administration, was associated with an 89% fall in brain intracellular glucose concentration.

The changes in brain produced by insulin-induced hypoglycemia were corrected when glucose was given intravenously in adequate amounts to raise plasma glucose to normal. In group III, the mean plasma level of glucose was 2.3±0.4 mM and mean plasma osmolality was 288±3 mosmol/kg, 208 min after insulin injection. 35 min after the intravenous injection of 5 ml or 13 ml of 2.8 M glucose solution to these animals, the levels of glucose in plasma were 6.9±0.9 and 24.7 ±3.7 mM, and plasma osmolalities were 297±4 and 304±2 mosmol/kg, respectively. Despite the differences in plasma glucose and osmolality, the brain content of water, osmoles, Na^+, K^+, and Cl^- were not significantly different, and thus, the data from the two subgroups have been pooled together.

The brain water content (385±4 g/100 g dry wt) was not significantly different from that of normal animals, and brain osmolality (308±4 mosmol/kg H_2O) was neither different from that of plasma (301±2 mosmol/kg) nor from that of brain of normal animals. The brain intracellular K^+ content (405±9 meq/kg intracellular dry wt) was not different from normal,

TABLE III

Effect of Insulin and Hypoxia on Brain Water and Solute

	Na^+	K^+	Cl^-	H_2O	Osmolality	Brain $^{35}SO_4^=$	[^{125}I]-Albumin	Glucose	Lactate	Glutamate	ECS
	meq/kg dry wt			*g/100 g dry wt*	*mosmol/kg H_2O*	%	%	*mmol/kg H_2O*			%
Normal, $n = 7$	237	409	174	372	300	4.7	2.2	2.9	5.5	10.7	22.1
	±5	±7	±2	±7	±8	±0.7	±0.2	±0.5	±0.5	±1.0	±0.4
Insulin, *35 min*, $n = 8$	238	440*	171	394‡	306	5.4	3.0	2.9	5.7	9.1	22.5
	±8	±4	±6	±8	±15	±0.8	±0.7	±0.4	±0.5	±0.7	±0.7
Insulin, *146 min*, $n = 7$	253*	439*	178	390‡	322*	4.6	2.3	2.1	5.8	8.1	21.9
	±4	±5	±5	±5	±3	±0.1	±0.1	±0.6	±0.3	±0.3	±0.3
Insulin, *208 min*, $n = 6$	254‡	444‡	191*	416§	298	5.4	2.7	1.0*	4.3‡	7.6‡	23.9*
	±6	±6	±4	±5	±6	±0.6	±0.3	±0.1	±0.3	±0.9	±0.5
Insulin, *208 min*, +glucose, $n = 10$	255‡	410	173	385	308	—	—	5.4‡	5.2	9.5	21.4
	±5	±10	±3	±4	±4	—	—	±0.8	±0.3	±0.4	±0.7
Hypoxia, *35 min*, $n = 8$	237	417	165	395‡	334*	5.8	3.8‡	4.3	18.4*	—	20.8
	±6	±7	±7	±8	±10	±0.7	±0.7	±0.7	±2.9	—	±0.7

Values are presented as Mean±SE.
* $P < 0.01$ vs. normal.
‡ $P < 0.05$ vs. normal.
§ $P < 0.001$ vs. normal.

although brain intracellular Na^+ (99±2 meq/kg intracellular dry wt) remained significantly greater than the normal value ($P < 0.01$). Brain glucose concentration exceeded normal values, while lactate was normal. The brain ECS, $^{35}SO_4^=$ space, and [^{125}I]albumin space were all normal (Table III).

After 35 min of hypoxia, with arterial P_{O_2} maintained at 23±1 mm Hg, plasma osmolality (311±3 mosmol/kg) and glucose (18.6±2.6 mM) were significantly greater than normal. Gross brain edema was present, as evidenced by bulging of the brain through a trephine opening in the skull. Brain osmolality (334 ±10 mosmol/kg H_2O) was significantly greater than that of plasma ($P < 0.05$) and than that of brain of normal rabbits ($P < 0.01$). Thus, there was an osmotic gradient between plasma and brain favoring movement of water into brain. Brain extracellular water content (103±2 g/100 g extracellular dry wt) was not different from normal, but intracellular water content (292±6 g/100 g intracellular dry wt) was significantly greater than normal ($P < 0.01$). Much of the increase in brain osmolality was due to a highly significant increase in brain lactate concentration (Table III). Despite the presence of hypoxia, the brain intracellular contents of Na^+ (76.5±1.9 meq/kg intracellular dry wt) and K^+ (412±6 meq/kg intracellular dry wt) were not different from normal values. The brain ECS and $^{35}SO_4^=$ spaces were normal, but the [^{125}I]albumin space of brain increased slightly but significantly ($P < 0.05$). Brain glucose concentration (4.3±0.7 mmol/kg H_2O) was significantly greater than normal ($P < 0.01$). Hypoxia did not produce any important alteration in the CSF concentration of Na^+, K^+, Cl^-, osmoles, or glucose, but CSF lactate (10.0±1.5 mmol/liter was significantly greater than normal.

The simultaneously measured osmolalities of brain and CSF were evaluated in 70 rabbits who had been maintained in a steady state for at least 2 h, and the results are shown in Fig. 3. Osmolalities of brain and CSF were similar throughout the entire range evalu-

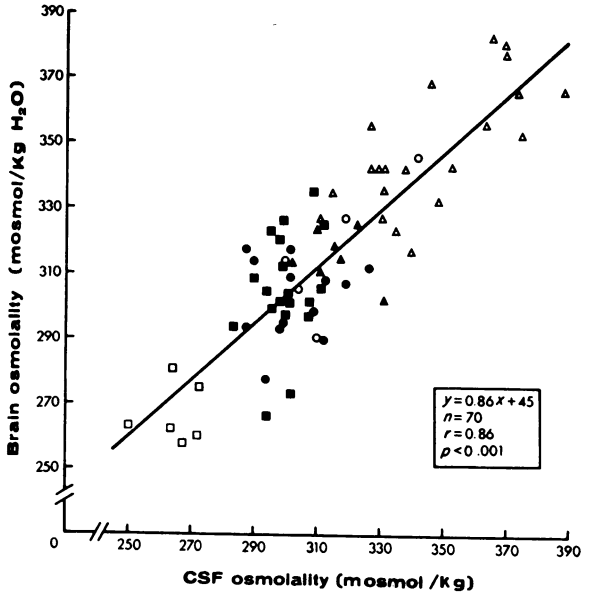

FIGURE 3 The relationship between osmolality of brain tissue vs. that of CSF in six groups of rabbits who had been maintained in a steady state for at least 2 h. The groups are: ●, normal; ▲, alloxan diabetes (10–16 days); △, glucose infusion (2–6 h); ○, hypoxia (2 h); ■, hypoglycemia (146–208 min); and □, water intoxication (2 h).

ated (250–390 mosmol/kg H_2O), with a highly significant correlation ($r = 0.86$, $t = 8.25$, $P < 0.001$).

DISCUSSION

The results of the present study demonstrate that insulin-induced hypoglycemia is associated with an increase in both intracellular and extracellular water content in brain. The increment in water content of brain appears to be secondary to a rise in brain osmolality due to increases in the brain content of K^+ and Na^+. The results show further, that as hypoglycemia progresses and blood glucose falls to very low levels, 208 min after the injection of insulin, the concentrations of glucose, lactate, and glutamate in brain decrease significantly. These changes are probably related to the occurrence of grand mal seizures and coma that develop early in the course of progressive insulin-induced hypoglycemia. It is of interest that the awake animals (group I) developed seizures 82–210 min after the injection of insulin (Fig. 3), but between seizures the animals were alert and responsive to stimuli. This time period coincides with the period in which cerebral edema was initially demonstrated in the hypoglycemic anesthetized animals (Table III). At a later time, 146–260 min after injection of insulin, the awake animals continued to have seizures but developed coma as well. At this time, the rabbits not only had cerebral edema, but also the concentration of substances providing metabolic fuels for brain activity had fallen to very low levels; these two events may explain the seizures and coma.

The increment in brain intracellular K^+ content occurred 35 min after the injection of insulin and at a time when the concentrations of glucose in plasma and brain were normal. This observation suggests a direct effect of insulin on K^+ transport into brain. Since the brain content of Na^+ and Cl^- also increased during the administration of insulin, it is possible that this hormone has a nonspecific effect on brain cell permeability allowing entry of various ions into the brain. The observation that the brain concentration of sulfate ($^{35}SO_4^=$) was not affected during the administration of insulin militates against such a postulate. The increase in brain tissue Cl^- was similar to the increase in brain extracellular Na^+ content (Table III), so that most of the increase in brain Cl^- was probably extracellular. The principal intracellular anions in brain are organic acids, lipid, and proteins (24), which can probably change their number of anionic sites in response to changes in intracellular pH, in a manner similar to the Cl^- shift in red blood cells (25).

The action of insulin causing an increase in brain K^+ content is probably on the cell membrane (26) and might occur at the brain cell-capillary interface or the CSF-brain cell interface. Two findings in our study suggest that insulin acts at the brain cell-capillary interface to promote uptake of K^+ by brain cells. First, insulin was not detected in CSF at a time when the increment in brain content of K^+ was maximal; second, the K^+ level in CSF did not change after insulin, while that in plasma fell substantially (Tables I and II). Such an effect of insulin on K^+ transport into brain is not surprising, since insulin has been shown to increase transport of K^+ into other cells, such as liver (27) and skeletal muscle (28, 29), and these effects are independent of the action of insulin on cellular uptake of glucose (11, 30).

Data shown in Fig. 2 suggest that most of the important changes in brain H_2O, K^+, and glucose which are temporally related to the occurrence of seizures and coma are intracellular. Such an interpretation is dependent upon the methodology used to evaluate brain ECS, and at the present time, there is considerable divergence of opinion as to the size of this compartment. However, when measured either by chemical markers or derived from measurement of cortical impedance, values for brain ECS of 12–25 g/100 wet wt are usually obtained (13, 20), and these are similar to results of the present study (Table III).

It is conceivable that the observed increase in brain water and K^+ content observed after insulin administration might be secondary to an increase in brain glycogen. One of the ways by which insulin may act to increase K^+ movement into skeletal muscle is by incorporation of K^+ and water into glycogen (29). However, previous studies from this laboratory have shown that insulin-mediated movement of K^+ and water into brain can occur in the absence of an increase in brain glycogen (9). Additionally, it has been shown that insulin appears to have virtually no effect on glucose uptake by the brain (31–33) and that in insulin-induced hypoglycemia, there is actually a fall in brain glycogen (34). Thus, it is unlikely that the observed increases in brain K^+ and water content are related to changes in brain glycogen.

It has also been suggested that when plasma glucose is lowered under certain conditions, an increase in brain sorbitol might contribute to the development of cerebral edema (35). However, brain sorbitol, even in hyperglycemic states, has never been shown to accumulate in osmotically significant quantities (36, 37). Since lowering of plasma glucose generally results in a decrement of brain and CSF sorbitol (9, 36), it is improbable that sorbitol accumulation had any important effect on the brain in the present study.

Results from in vitro studies on brain tissue slices have suggested that insulin may not affect brain K^+ transport (33). It is difficult to extrapolate the results

of in vitro studies on brain to the in vivo situation. In vitro studies involve removal of the blood-brain barrier, alterations in brain metabolism, and damage to brain caused by its removal and subsequent slicing. It has been shown, for example, that within only a few seconds of removal of the brain from an intact animal, there is an increase in its osmolality (18) and a fall in glucose and a rise in lactate concentrations (38). Furthermore, changes in the plasma concentration of other glucoregulatory hormones that may rise during insulin administration—epinephrine, glucagon, growth hormone, cortisol—could account for an appreciable difference in such comparative observations.

The increase in brain Na^+ after insulin administration was surprising. Insulin has been shown to actively alter the efflux of Na^+ (39–41) in several cell systems by a mechanism which is not mediated through the action of Na^+-K^+ ATPase (42). In addition, transport of glucose and Na^+ into cells has been shown to be linked in several different cell systems (43, 44), and insulin has been shown to increase reabsorption of Na^+ by the kidney both in normal individuals (45) and in those with diabetes mellitis (46). Thus, it is conceivable that insulin could affect cellular uptake of Na^+ in brain. Previous work from this laboratory has suggested that insulin might enhance entry of K^+ and Na^+ into brain of normal animals (9) and that hyperglycemia (plasma glucose $>$ 14 mM) might inhibit such an effect. Data from the present investigation not only appear to confirm these initial impressions, but also suggest that hyperglycemia can reverse most of the abnormalities of electrolyte transport into brain associated with excess insulin administration (Fig. 2). This finding is consistent with the clinical observations that the symptoms of insulin-induced hypoglycemia are corrected by the administration of intravenous glucose. The rise in blood glucose may antagonize the effect of insulin on electrolyte transport into brain and hence correct the abnormalities that lead to brain edema.

Further support for the effect of insulin on the water and electrolyte content of brain is found in the clinical observations that while insulin-induced hypoglycemia is often accompanied by seizure (1–3, 47) severe hypoglycemia (plasma glucose $<$ 2.5 mM) which was not caused by excess insulin is not usually associated with coma and seizures (48, 49). When such hypoglycemic but asymptomatic subjects are given intravenous insulin, neurologic symptoms may rapidly develop, even in the absence of a further fall in the levels of plasma glucose (2).

It is theoretically possible that the brain edema observed in the hypoglycemic animals was a consequence of altered capillary permeability, with leakage of intravascular contents into the interstitium (50). The finding that [^{125}I]albumin space of brain was normal in the hypoglycemic animals makes this possibility remote.

In contrast to hypoglycemia, we found (Table III), as have others (51), that hypoxia of up to 3-h duration did not affect the electrolyte content of brain. Brain osmolality increased largely due to an increase in brain concentration of lactate (Table III) and probably other organic acids as well (52). The rise in brain osmolality was largely responsible for movement of water into brain and the production of brain edema. During hypoxia, the intracellular pH of brain falls significantly (52), while brain lactate content increases, with no change in brain Na^+ or K^+ content. This strongly suggests that the increased brain lactate is in the form of lactic acid, rather than Na^+ or K^+ lactate. Additionally, the [^{125}I]albumin space of brain in the hypoxic animals increased from 2.2±0.2 to 3.8±0.7 g/100 g wet wt ($P < 0.05$), which suggests that an increase in capillary permeability with leakage of intravascular contents into the interstitium occurred during hypoxia. This change may partly contribute to brain edema that occurs with hypoxia.

It appears from the results of the present study that the onset of coma late in the course of hypoglycemia is a consequence, at least in part, of both brain edema and depletion of energy-supplying substrates in brain. However, seizures observed soon after the onset of hypoglycemia are probably related to increased transport of K^+ and Na^+ into brain cells, with resultant increase in brain osmolality and water content. Both hypoglycemia and hypoxia are associated with cerebral edema, but the pathophysiologic events that lead to the rise in the water content of brain in these two conditions are different.

ACKNOWLEDGMENTS

This study was supported by Grants No. AM16747 and RR05468 from the United States Public Health Service.

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