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RECOVERY AFTER HYPOGLYCEMIC BRAIN INJURY

ACTION OF SOME BIOLOGICAL SUBSTANCES ON THE CEREBRAL METABOLISM

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Abstract—In artificially ventilated beagle dogs a severe hypoglycemic condition was induced by insulin injection, while the posthypoglycemic recovery was induced by glucose treatment at the end of a 20-min period of spontaneous electroencephalographic silence. The motor area of the cerebral cortex was analyzed for glycolytic metabolites, related amino acids, energy mediators, fatty acids, phospholipids and free fatty acids. The effects on the posthypoglycemic recovery of a intracarotid infusion with some agents (i.e. uridine, cytidine, DL-carnitine, DL-acetylcarnitine, papaverine) were tested. Severe hypoglycemia induced an extensive derangement of the brain metabolism, with partial restitution during the posthypoglycemic recovery. During this condition, the intracarotid perfusion with some biological pyrimidines (uridine, cytidine) interfered with the glycolytic and amino acid metabolites, inducing a decrease in glucose, pyruvate and lactate contents, and an increase in succinate, alanine and glutamine cerebral concns. The lipid carriers (DL-carnitine, DL-acetylcarnitine) interfered with the fatty acid degradation inducing a magnification of the decrease in the individual (palmitic acid, oleic acid) and total fatty acids, the vasodilating agent (papaverine) being practically inactive.

Prolonged hypoglycemia has been shown to cause brain damage [1–13]. The time course of the neuronal alterations involves a transition from "microvacuolation" (by swelling of mitochondria?) to "ischemic cell change", with terminal "dissolution" of the neuron. The selective vulnerability denotes the preferential localization of damaged neurons to the cerebral cortex (particularly the small pyramidal neurons in layer 5), the hippocampus and the striatum [8]. The hypoglycemic derangements have the same localization to selective areas as occurs in cerebral hypoxia [9], even if some metabolic alterations occurring in hypoglycemia are different from those occurring in hypoxia, e.g. absence of cellular lactacidosis.

During hypoglycemia the cerebral blood flow is unchanged or increased [14–17]. The moderate hypoglycemic condition is associated with a normal or even increased cerebral metabolic rate for oxygen (CMR_{O_2}), while during severe hypoglycemia the CMR_{O_2} is normal or decreased [14–18]. In any case, a substantial decrease in the cerebral metabolic rate for glucose (CMR_{Gl}) may be concomitant to a normal CMR_{O_2} [19, 20]. Therefore, the hypoglycemic condition reduces the utilization of glucose to a greater extent than oxygen, the "oxygen:glucose" uptake ratio being increased to above 6, suggesting that other exogenous substrates than glucose are taken up from the circulation and utilized for oxidation or that other endogenous substrates than glucose are oxidized by brain tissue. During hypoglycemia there is both a reduction in the phospholipid content and an accumulation of free fatty acids in the cerebral cortex, the relatively largest increase occurring in the concn of arachidonic acid, which could serve as a substrate for increased prostaglandin synthesis. Severe hypoglycemia is accompanied by cessation of the EEG with decreases in both creatine phosphate and ATP, and increases in ADP and AMP cerebral contents [19, 21–23].

The present experiments were performed in order to study both the substrate utilization and the influence of some biological substances (i.e. cytidine, uridine, DL-carnitine, DL-acetylcarnitine) on the recovery of brain metabolism following pronounced hypoglycemia, with an isoelectric EEG for 20 min. Recovery was induced for 10, 20 or 40 min by glucose injection. Under these conditions definite modifications take place in glycolytic metabolites, related amino acids, energy mediators, fatty acids and phospholipids [4, 5, 23]. However, the role of the tested agents in the regulation of cerebral biochemical function during the recovery after hypoglycemic brain injury has not yet been investigated. Concerning uridine and cytidine, it is well known that some biological pyrimidines (e.g. uridine, cytidine) are related to cerebral function. In experiments carried out in isolated cat brain [24] it has been shown that the addition of cytidine and uridine to the perfusion blood keeps the brain in a good functional state. Moreover, the addition of nucleosides after 1 hr of perfusion shifts carbohydrate metabolism toward normal, while decreasing brain lactate. Other investigators [25–27] have evaluated in several animal species the effect induced by nucleosides, nucleotides and heterocyclic pyrimidinic bases on: (a) bipolar

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electrocorticograms utilizing electrodes placed on the olfactory bulb and posterior forebrain of each hemisphere; (b) transport through the blood–brain barrier of cytosine, uracyl and their nucleosides and nucleotides; and (c) the interaction with barbiturate narcosis, or with brain stem analeptic- and penicillin-induced seizures. The investigations, performed using different experimental techniques, have shown that uracyl derivatives are the most active in potentiating barbiturate narcosis and in antagonizing analeptic- or penicillin-induced seizures. These events at brain level have also been ascribed to the structural similarity between barbiturates and pyrimidines. Indeed, the group –CO–NH–CO– is present in all pyrimidine nucleosides and nucleotides. These observations have suggested that pyrimidine nucleosides and nucleotides play a role in the regulation of cerebral nervous system function [26].

Concerning carnitine and acetylcarnitine, investigations over the past few years have indicated that these substances are the requisite carriers for most transmembrane movements of long-chain fatty acids [28, 29]. Therefore, carnitine and/or its esters may be required for the normal function of various organs, e.g. carnitine deficiency leads to intracellular lipid accumulation and muscle myopathy [30–32], while carnitine depletion from the myocardium reduces fatty acid oxidation and impairs myocardial function [33]. Pronounced changes in the tissue contents of carnitine and its esters occur in response to a variety of alterations in the metabolic state [34].

METHODS

Animals and anesthesia. The experiments were carried out on female beagle dogs aged 1.5 years and weighing 8.5–12.3 kg, maintained under standard environmental conditions and fed a standard diet as pellets, with water *ad lib*. The animals were fasted for 24 hr before the experiments, but had free access to water. In devising the anesthetic procedures we aimed at providing sufficient analgesia, yet avoiding marked changes in cerebral metabolic rate [35–41]. This was achieved as follows: the surgical procedure was performed on animals preanesthetized with urethane (0.4 g/kg i.p.). Anesthesia was induced and maintained during the surgical procedure by chloralose (20 or 40 mg/kg i.v.) and by intermittent nitrous oxide inhalation. During the operative procedure and the experiment, the animals were treated with an i.v. injection of gallamine triethiodide (2–3 mg/kg) to permit unresisted artificial respiration and to maintain a constant $PaCO_2$ (35–40 mmHg). The dogs were artificially ventilated with air.

Operative procedure. The procedure [42] consisted mainly in isolating both common carotid arteries, while the zygomatic, maxillary, auricolar and supraorbital vessels were all occluded by ligature or compression. The animal's head was fixed to a head-holder with the confluence of cerebral venous sinuses approximately 10 cm higher than the heart. After bilateral, longitudinal incisions of skin and muscles, two holes were made in the frontal–parietal bones of the skull without damaging the subjacent cortex. A plastic funnel was fitted into each hole, leaving the dura intact beneath them. Both muscle and skin were sutured tightly around the funnels. These were sealed with rubber stoppers and insulated to prevent untoward ambient heat and pressure exchange. One femoral artery was cannulated for blood pressure recording and for sampling of blood. In fact, repeated arterial samples were collected directly in liquid nitrogen for later analyses of glucose. One femoral vein was cannulated for injections. Electrical activity of the brain, as portrayed by electroencephalography, was used to determine both the degree of anesthesia and the intensity of depression induced by severe hypoglycemia. The rectal temp was monitored with a telethermistor, and intermittent heating was applied to keep the dog's temp at approximately 37°. Physiological parameters were recorded using a 12-channel recorder (Physioscript EE12, Schwarzer).

Experimental plan. There were various groups of dogs. One was a control group of animals maintained under artificial ventilation for 120 min before cerebral tissue was sampled (normoglycemic untreated dogs). In other groups, dogs were kept at a steady state until the EEG became isoelectric because of an intraperitoneal injection of insulin at a dose of 40 I.U.kg^{-1}. In fact, the beginning of the period of severe hypoglycemia was defined as the time when spontaneous EEG activity disappeared. The spontaneous EEG activity disappeared when blood glucose concns decreased to values below 1 $\mu mole \cdot g^{-1}$.

The dogs were then maintained for 10 or 20 min with an isoelectric EEG before brain was sampled (hypoglycemic untreated or drug-treated dogs). During the isoelectric periods, no value of the blood glucose level was higher than 1.08 $\mu moles \cdot g^{-1}$ and the lowest was 0.38 $\mu moles \cdot g^{-1}$, the mean ± S.E.M. of 69 experiments being 0.82 ± 0.04 $\mu moles \cdot g^{-1}$. Other groups were allowed a 10-, 20- or 40-min recovery period, following an isoelectric period of 20 min (posthypoglycemic untreated or drug-treated dogs). Recovery was induced by an i.v. glucose injection (4 $ml \cdot kg^{-1}$ of a 20% glucose solution) and maintained by an i.v. glucose perfusion (0.2 $ml \cdot min^{-1} \cdot kg^{-1}$ of a 20% glucose in Krebs–Henseleit solution). In this way, during recovery no single value of the blood glucose level fell below 3.07 $\mu moles \cdot g^{-1}$, the mean ± S.E.M. of 45 experiments being 5.76 ± 0.12 $\mu moles \cdot g^{-1}$.

Therefore metabolites were measured in the cortical portion of the motor area of the brain: (a) in control condition; (b) after 10 min of severe hypoglycemia; (c) after 20 min of severe hypoglycemia (recovery time = 0 min); and (d) after 10 min (recovery time = 10 min) or after 20 min (recovery time = 20 min) or after 40 min (recovery time = 40 min) of posthypoglycemic recovery following an isoelectric period of 20 min. The effect of the intracarotid perfusion with some agents was tested at recovery times of 0, 10, 20 or 40 min, respectively. In fact, an intracarotid perfusion (via the superior thyroid arteries, at a rate of 0.1 $ml \cdot min^{-1} \cdot kg^{-1}$) was performed: for 20 min (during hypoglycemia) or for 20 min (during hypoglycemia) plus 10, 20 or 40 min (during a posthypoglycemic recovery of 10, 20 or 40 min, respectively) with: (a) saline solution; (b)

uridine (1.25×10^{-2} M); (c) cytidine (1.25×10^{-2} M); (d) DL-carnitine (5×10^{-2} M); (e) DL-acetylcarnitine (5×10^{-2} M); and (f) papaverine (5×10^{-4} M).

Withdrawal of cerebral samples and analytical techniques. Carbohydrate and amino acid metabolites were evaluated in the cortical tissue from one motor area while lipid metabolites were evaluated in the cortical tissue from the contralateral motor area of the same dog. At a set time, a portion of the motor area of the brain was frozen *in situ* by liquid nitrogen and removed from the plastic funnels fitted into the skull. This portion of the brain was cut *in situ* by means of a rotating hollow tube during liquid nitrogen perfusion.

For the analytical procedure, the cerebral extract from this cortical side was used [43, 44] to evaluate: glycolytic metabolites (glycogen, glucose, pyruvate, lactate), tricarboxylic acid cycle intermediates (α-ketoglutarate, succinate), related amino acids (glutamate, glutamine, aspartate, alanine, gamma-aminobutyrate, ammonia) and energy mediators (ATP, ADP, AMP, creatine phosphate), the energy charge potential [45] being defined as ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]). The cortical extract from the other motor area of the same dog was used [46, 47] to evaluate: fatty acids [total, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), arachidonic acid (20:4), docosahexenoic acid (22:6)], phospholipids (total, choline phosphoglycerides, inositol plus serine phosphoglycerides, ethanolamine phosphoglycerides), and total free fatty acids.

Statistical analysis. As regards the posthypoglycemic recovery, the data concern six treatments (NaCl, papaverine, uridine, cytidine, DL-carnitine, DL-acetylcarnitine), each of them being related to four groups of dogs. In fact the withdrawal of brain tissue was performed with the surface freezing technique at four different times, namely 0, 10, 20 and 40 min after the beginning of the restitution. We utilized in total 29 data for each parameter (cerebral concn of glycogen, glucose etc.), and to these data we applied the analysis of variance (Anova) for factorial experiments (six-level treatment factor and four-level time factor). This Anova collects F values for the difference "between treatments" or "between times" and, particularly, F values related to the interaction "treatments by times". The 5 df of the Anova "between treatments" have been distributed according to the scheme: (a) NaCl vs other treatments; (b) papaverine vs other treatments (NaCl excluded); (c) uridine vs other treatments (NaCl and papaverine excluded); (d) cytidine vs DL-carnitine and DL-acetylcarnitine; and (e) DL-carnitine vs DL-acetylcarnitine.

RESULTS

Alterations occurring during hypoglycemia

For the periods of 10 and 20 min of isoelectric EEG (Tables 1 and 2), there was in the motor area of the cerebral cortex of beagle dogs: (a) an extensive drop in the contents of glycolytic metabolites (glycogen, glucose, pyruvate, lactate) and α-ketoglutarate; (b) a marked breakdown of ATP and creatine

Table 2. Motor area of cerebral cortex of beagle dogs treated with saline solution: contents of fatty acids, phospholipids and free fatty acids during insulin-induced severe hypoglycemia and posthypoglycemic recovery period

Experimental conditions	Fatty acids						Phospholipids				Free fatty acids
	Palmitic acid	Stearic acid	Oleic acid	Arachidonic acid	Docosahexenoic acid	Total	Choline phosphoglycerides	Inositol + serine phosphoglycerides	Ethanolamine phosphoglycerides	Total	
Control	32.2 ± 2.2	29.6 ± 2.1	30.5 ± 1.6	14.2 ± 1.7	22.0 ± 2.1	128.3 ± 5.6	28.1 ± 1.4	12.6 ± 1.6	30.5 ± 1.5	76.2 ± 2.2	0.21 ± 0.03
Severe hypoglycemia											
10 min	29.1 ± 1.6	27.4 ± 1.4	26.2 ± 0.6	12.1 ± 0.5	21.5 ± 1.2	115.6 ± 1.4	25.1 ± 0.5	11.4 ± 0.4	27.1 ± 2.3	70.3 ± 2.8	0.86 ± 0.03*
20 min (RT = 0)	27.8 ± 1.1	23.9 ± 0.6*	23.7 ± 0.8*	11.0 ± 0.5*	21.1 ± 1.7	113.2 ± 2.1*	23.6 ± 0.9*	11.6 ± 1.0	26.4 ± 1.5	68.2 ± 1.2*	1.03 ± 0.06*
Posthypoglycemic recovery											
10 min (RT = 10)	28.1 ± 1.3	24.2 ± 1.9*	23.1 ± 1.6*	11.9 ± 1.4	21.6 ± 1.6	114.0 ± 2.1*	22.8 ± 1.3*	11.4 ± 1.6	25.5 ± 1.9	69.5 ± 2.9	1.02 ± 0.05*
20 min (RT = 20)	26.1 ± 2.1	21.0 ± 1.0*	25.7 ± 2.1	10.5 ± 1.0*	22.3 ± 0.9	111.5 ± 4.2*	20.9 ± 2.0*	10.5 ± 1.2	28.8 ± 2.9	66.3 ± 2.3*	0.50 ± 0.08*†
40 min (RT = 40)	28.3 ± 1.5	22.8 ± 1.3*	24.6 ± 1.2*	12.3 ± 1.6	22.8 ± 1.5	117.6 ± 1.8*	21.7 ± 0.7*	11.0 ± 0.8	27.3 ± 1.4	67.9 ± 1.0*	0.26 ± 0.02†

The values are expressed as μmoles · (g cortex wet wt)$^{-1}$ (means ± S.E.M.).
RT = recovery time (min).
* = differs from control conditions. Anova test ($P < 0.05$): N = 5 (control conditions) or 4 (other conditions).
† = differs from recovery time 0 (RT = 0)

Table 1. Motor area of cerebral cortex of beagle dogs treated with saline solution: contents of carbohydrate metabolites,

Experimental conditions	Glycogen	Glucose	Lactate	Pyruvate	Lactate/ pyruvate	α-Ketoglutarate	Succinate	Glutamate
Control	3.05 ± 0.15	3.56 ± 0.21	1.65 ± 0.09	0.114 ± 0.004	14.37 ± 0.35	0.168 ± 0.010	0.438 ± 0.014	10.75 ± 0.45
Severe hypoglycemia								
10 min	0.21 ± 0.05*	0.15 ± 0.04*	0.43 ± 0.08*	0.036 ± 0.004*	12.41 ± 2.64	0.051 ± 0.008*	0.510 ± 0.024*	5.62 ± 0.40*
20 min (RT = 0)	0.08 ± 0.01*	0.10 ± 0.03*	0.22 ± 0.04*	0.031 ± 0.003*	7.79 ± 1.96*	0.025 ± 0.003*	0.598 ± 0.038*	3.84 ± 0.26*
Posthypoglycemic recovery								
10 min (RT = 10)	0.14 ± 0.03*	1.72 ± 0.17*†	1.37 ± 0.12†	0.086 ± 0.08*†	16.00 ± 0.62*†	0.063 ± 0.010*†	0.675 ± 0.021*	6.95 ± 0.34*†
20 min (RT = 20)	0.17 ± 0.02*†	2.65 ± 0.23*†	1.75 ± 0.10†	0.118 ± 0.009†	15.03 ± 1.19†	0.093 ± 0.009*†	0.750 ± 0.031*†	8.46 ± 0.36*†
40 min (RT = 40)	0.33 ± 0.03*†	3.32 ± 0.22†	2.05 ± 0.08*†	0.148 ± 0.010*†	13.97 ± 0.83†	0.138 ± 0.018†	0.706 ± 0.024*	10.08 ± 0.41†

The values are expressed as μmoles·(g cortex wet wt)$^{-1}$ (means ± S.E.M.).
RT = recovery time (min).
* = differs from control condition
† = differs from recovery time 0 (RT = 0)
} Anova test ($P < 0.05$): N = 5 (control conditions) or 4 (other conditions).

Table 3. Motor area of cerebral cortex of beagle dogs: contents of carbohydrate metabolites, amino acids and energy mediators after 10 min of

Treatment	Glycogen	Glucose	Lactate	Pyruvate	Lactate/ pyruvate	α-Ketoglutarate	Succinate	Glutamate
Saline solution	0.14 ± 0.03	1.72 ± 0.17	1.37 ± 0.12	0.086 ± 0.008	16.00 ± 0.62	0.063 ± 0.010	0.675 ± 0.021	6.95 ± 0.34
Papaverine	0.10 ± 0.04	1.35 ± 8.12	1.21 ± 0.13	0.095 ± 0.006	12.97 ± 2.39	0.056 ± 0.007	0.635 ± 0.045	6.41 ± 0.42
Uridine	0.16 ± 0.06	1.19 ± 0.08*	0.96 ± 0.107	0.051 ± 0.007*	18.79 ± 0.96	0.056 ± 0.003	0.785 ± 0.044*	6.08 ± 0.22
Cytidine	0.15 ± 0.03	1.48 ± 0.11	1.20 ± 0.09	0.071 ± 0.006	16.88 ± 0.19	0.060 ± 0.005	0.793 ± 0.035*	7.15 ± 0.21
DL-Carnitine	0.18 ± 0.04	2.03 ± 0.08	1.50 ± 0.17	0.091 ± 0.013	16.61 ± 0.58	0.078 ± 0.005	0.621 ± 0.006	7.46 ± 0.47
DL-Acetylcarnitine	0.20 ± 0.05	2.12 ± 0.16	1.61 ± 0.17	0.100 ± 0.012	16.16 ± 0.54	0.083 ± 0.020	0.610 ± 0.059	7.65 ± 0.57

The values are expressed as μmoles·(g cortex wet wt)$^{-1}$ (means ± S.E.M.).
* = differs from saline solution treated dogs: Anova test ($P < 0.05$): N = 4 (saline solution treated animals) or 3 (drug-treated animals).

Table 4. Motor area of cerebral cortex of beagle dogs: contents of carbohydrate metabolites, amino acids and energy mediators after 20 min of

Treatment	Glycogen	Glucose	Lactate	Pyruvate	Lactate/ pyruvate	α-Ketoglutarate	Succinate	Glutamate
Saline solution	0.17 ± 0.02	2.65 ± 0.23	1.75 ± 0.10	0.118 ± 0.009	15.03 ± 1.19	0.093 ± 0.0	0.750 ± 0.031	8.46 ± 0.36
Papaverine	0.12 ± 0.04	1.93 ± 0.29	2.03 ± 9.20	0.136 ± 0.030	15.70 ± 1.70	0.078 ± 0.016	0.695 ± 0.036	7.86 ± 0.36
Uridine	0.22 ± 0.05	1.86 ± 0.08*	1.29 ± 0.04*	0.075 ± 0.010*	17.93 ± 2.54	0.084 ± 0.005	0.852 ± 0.022*	7.95 ± 0.46
Cytidine	0.24 ± 0.05	2.28 ± 0.12	1.49 ± 0.21	0.099 ± 0.012	15.08 ± 0.62	0.089 ± 0.011	0.839 ± 0.013*	7.82 ± 0.38
DL-Carnitine	0.21 ± 0.05	2.91 ± 0.34	2.06 ± 0.19	0.129 ± 0.017	16.20 ± 1.01	0.112 ± 0.017	0.715 ± 0.038	7.96 ± 0.30
DL-Acetylcarnitine	0.26 ± 0.05	3.00 ± 0.19	2.10 ± 0.19	0.122 ± 0.007	17.10 ± 0.81	0.120 ± 0.023	0.700 ± 0.039	7.45 ± 0.87

The values are expressed as μmoles·(g cortex wet wt)$^{-1}$ (means ± S.E.M.).
* = differs from saline solution treated dogs: Anova test ($P < 0.05$); N = 4 (saline solution treated animals) or 3 (drug-treated animals).

Table 5. Motor area of cerebral cortex of beagle dogs: contents of carbohydrate metabolites, amino acids and energy mediators after 40 min of

Treatment	Glycogen	Glucose	Lactate	Pyruvate	Lactate/ pyruvate	α-Ketoglutarate	Succinate	Glutamate
Saline solution	0.33 ± 0.03	3.32 ± 0.22	2.05 ± 0.08	0.148 ± 0.010	13.97 ± 0.83	0.138 ± 0.018	0.706 ± 0.024	10.08 ± 0.41
Papaverine	0.23 ± 0.05	2.18 ± 0.11*	2.37 ± 0.32	0.132 ± 0.010	18.51 ± 4.06	0.121 ± 0.020	0.637 ± 0.049	9.80 ± 0.92
Uridine	0.34 ± 0.06	2.65 ± 0.28*	1.68 ± 0.10*	0.110 ± 0.010*	15.34 ± 0.46	0.130 ± 0.004	0.798 ± 0.012*	9.61 ± 0.27
Cytidine	0.40 ± 0.05	3.07 ± 0.18	1.85 ± 0.08	0.129 ± 0.007	14.48 ± 1.51	0.145 ± 0.013	0.781 ± 0.019	10.14 ± 0.60
DL-Carnitine	0.38 ± 0.05	3.85 ± 0.30	2.38 ± 0.24	0.144 ± 0.014	16.49 ± 0.12	0.151 ± 0.011	0.655 ± 0.07	11.00 ± 0.33
DL-Acetylcarnitine	0.42 ± 0.10	3.92 ± 0.28	2.29 ± 0.21	0.157 ± 0.012	14.49 ± 0.27	0.162 ± 0.020	0.632 ± 0.055	10.53 ± 0.78

The values are expressed as μmoles·(g cortex wet wt)$^{-1}$ (means ± S.E.M.).
* = differs from saline solution treated dogs: Anova test ($P < 0.05$): N = 4 (saline solution treated animals) or 3 (drug-treated animals).

phosphate, consistent with an increase in ADP and AMP and resulting in an extensive decrease in the cerebral energy charge potential; (c) an extensive accumulation of ammonia and aspartate, with reductions in the contents of the other amino acids tested (glutamate, glutamine, alanine, gamma-aminobutyrate); (d) a slight but evident decrease in total fatty acid content as well as in some individual fatty acid concns (stearic acid, oleic acid, arachidonic acid); (e) a slight but sustained decrease in total phospholipids as well as in some individual phospholipid classes (choline phosphoglycerides); and (f) a marked increase in total free fatty acid content. In any case, the prolongation of the isoelectric period from 10 to 20 min further (but moderately) exaggerated the cerebral biochemical derangement induced by severe hypoglycemia.

Events occurring during posthypoglycemic recovery

For the periods of 10, 20 and 40 min of recovery

amino acids and energy mediators during insulin-induced severe hypoglycemia and posthypoglycemic recovery period

Glutamine	Aspartate	Alanine	Gamma-aminobutyrate	Ammonia	ATP	ADP	AMP	E.C.P.	Creatine phosphate
5.76 ± 0.34	3.12 ± 0.21	0.506 ± 0.012	2.14 ± 0.13	0.34 ± 0.02	2.51 ± 0.06	0.44 ± 0.02	0.06 ± 0.01	0.907 ± 0.003	4.66 ± 0.28
1.38 ± 0.14*	9.36 ± 1.19*	0.375 ± 0.023*	1.78 ± 0.12	2.67 ± 0.25*	1.24 ± 0.08*	0.92 ± 0.05*	0.39 ± 0.05*	0.666 ± 0.013*	1.01 ± 0.07*
0.65 ± 0.05*	14.82 ± 1.07*	0.282 ± 0.014*	1.08 ± 0.07*	3.15 ± 0.20*	0.95 ± 0.09*	0.90 ± 0.05*	0.47 ± 0.04*	0.602 ± 0.023*	0.57 ± 0.08*
1.48 ± 0.10*†	9.96 ± 0.62*†	0.470 ± 0.026†	1.38 ± 0.13*	2.16 ± 0.16*†	1.42 ± 0.04*†	0.68 ± 0.06*†	0.31 ± 0.04*†	0.730 ± 0.007*†	1.61 ± 0.12*†
2.08 ± 0.15*†	7.36 ± 0.47*†	0.638 ± 0.034*†	1.68 ± 0.10*†	1.36 ± 0.17*†	1.64 ± 0.06*†	0.54 ± 0.07†	0.22 ± 0.03*†	0.795 ± 0.007*†	2.47 ± 0.25*†
2.76 ± 0.19*†	3.62 ± 0.37†	0.820 ± 0.051*†	2.04 ± 0.11†	0.46 ± 0.07†	1.98 ± 0.11*†	0.41 ± 0.04†	0.09 ± 0.02†	0.879 ± 0.012*†	4.06 ± 0.17†

posthypoglycemic recovery (recovery time 10 min) and treatment for 20 plus 10 min with an intracarotid perfusion (0.1 $ml \cdot kg^{-1} \cdot min^{-1}$) with saline solution or drug solution

Glutamine	Aspartate	Alanine	Gamma-aminobutyrate	Ammonia	ATP	ADP	AMP	E.C.P.	Creatine phosphate
1.48 ± 0.10	9.96 ± 0.62	0.470 ± 0.026	1.38 ± 0.13	2.16 ± 0.16	1.42 ± 0.04	0.68 ± 0.06	0.31 ± 0.04	0.730 ± 0.007	1.61 ± 0.12
1.25 ± 0.15	8.95 ± 0.58	0.425 ± 0.031	1.22 ± 0.14	2.48 ± 1.19	1.37 ± 0.10	0.59 ± 0.10	0.38 ± 0.05	0.713 ± 0.037	1.38 ± 0.10
2.36 ± 0.28*	11.30 ± 1.64	0.588 ± 0.036*	1.67 ± 0.10	1.70 ± 0.34	1.51 ± 0.05	0.62 ± 0.09	0.30 ± 0.05	0.750 ± 0.005	1.46 ± 0.13
1.81 ± 0.09	10.52 ± 0.80	0.535 ± 0.027	1.50 ± 0.11	1.93 ± 0.15	1.46 ± 0.07	0.73 ± 0.04	0.36 ± 0.03	0.715 ± 0.006	1.57 ± 0.11
1.70 ± 0.10	10.73 ± 1.50	0.410 ± 0.032	1.19 ± 0.17	2.48 ± 0.27	1.51 ± 0.08	0.72 ± 0.16	0.30 ± 0.04	0.741 ± 0.034	1.45 ± 0.16
1.78 ± 0.19	11.08 ± 0.91	0.421 ± 0.045	1.10 ± 0.22	2.54 ± 0.33	1.46 ± 0.07	0.57 ± 0.12	0.27 ± 0.03	0.759 ± 0.030	1.55 ± 0.18

posthypoglycemic recovery (recovery time 20 min) and treatment for 20 plus 20 min with an intracarotid perfusion (0.1 $ml \cdot kg^{-1} \cdot min^{-1}$) with saline solution or drug solution

Glutamine	Aspartate	Alanine	Gamma-aminobutyrate	Ammonia	ATP	ADP	AMP	E.C.P.	Creatine phosphate
2.08 ± 0.15	7.36 ± 0.47	0.638 ± 0.034	1.68 ± 0.10	1.36 ± 0.17	1.64 ± 0.06	0.54 ± 0.07	0.22 ± 0.03	0.795 ± 0.007	2.47 ± 0.25
1.95 ± 0.16	6.66 ± 0.26	0.583 ± 0.025	1.39 ± 0.11	1.71 ± 0.23	1.52 ± 0.11	0.66 ± 0.05	0.29 ± 0.04	0.748 ± 0.028	2.00 ± 0.10
3.42 ± 0.21*	8.95 ± 1.09	0.734 ± 0.028*	1.85 ± 0.06	0.70 ± 0.04*	1.57 ± 0.07	0.59 ± 0.07	0.25 ± 0.04	0.773 ± 0.026	2.30 ± 0.12
2.41 ± 0.18	8.06 ± 0.42	0.721 ± 0.009*	1.76 ± 0.19	1.09 ± 0.06	1.54 ± 0.13	0.63 ± 0.06	0.30 ± 0.02	0.748 ± 0.017*	2.52 ± 0.22
2.43 ± 0.24	6.83 ± 0.28	0.576 ± 0.036	1.46 ± 0.17	1.61 ± 0.16	1.53 ± 0.10	0.59 ± 0.10	0.26 ± 0.06	0.767 ± 0.029	2.12 ± 0.20
2.58 ± 0.36	7.15 ± 0.17	0.561 ± 0.054	1.53 ± 0.10	1.52 ± 0.14	1.60 ± 0.05	0.61 ± 0.07	0.20 ± 0.03	0.790 ± 0.006	2.38 ± 0.08

posthypoglycemic recovery (recovery time 40 min) and treatment for 20 plus 40 min with an intracarotid perfusion (0.1 $ml \cdot kg^{-1} \cdot min^{-1}$) with saline solution or drug solution

Glutamine	Aspartate	Alanine	Gamma-aminobutyrate	Ammonia	ATP	ADP	AMP	E.C.P.	Creatine phosphate
2.76 ± 0.19	3.62 ± 0.37	0.820 ± 0.051	2.04 ± 0.11	0.46 ± 0.07	1.98 ± 0.11	0.41 ± 0.04	0.09 ± 0.02	0.879 ± 0.012	4.06 ± 0.17
2.41 ± 0.28	3.03 ± 0.35	0.630 ± 0.047*	1.78 ± 0.21	0.70 ± 0.08	1.72 ± 0.09	0.53 ± 0.05	0.17 ± 0.03	0.818 ± 0.013*	3.80 ± 0.18
4.40 ± 0.25*	4.15 ± 0.69	0.937 ± 0.027	2.25 ± 0.12	0.30 ± 0.04	2.00 ± 0.07	0.39 ± 0.03	0.13 ± 0.02	0.869 ± 0.011	3.83 ± 0.16
3.38 ± 0.22	4.00 ± 0.18	0.909 ± 0.029	2.00 ± 0.13	0.40 ± 0.07	1.93 ± 0.06	0.45 ± 0.05	0.15 ± 0.04	0.852 ± 0.023	4.15 ± 0.20
2.50 ± 0.28	3.42 ± 0.30	0.766 ± 0.039	1.81 ± 0.14	0.53 ± 0.12	1.83 ± 0.12	0.46 ± 0.06	0.13 ± 0.02	0.853 ± 0.008	3.75 ± 0.47
2.43 ± 0.25	3.71 ± 0.09	0.739 ± 0.048	1.71 ± 0.15	0.42 ± 0.08	1.85 ± 0.07	0.38 ± 0.04	0.12 ± 0.02	0.867 ± 0.006	3.63 ± 0.12

following an isoelectric period of 20 min (Tables 1 and 2), there was in the motor area of the cerebral cortex of beagle dogs: (a) a progressive and marked restoration of glycolytic metabolites (glucose, pyruvate, lactate); (b) a slight rate of glycogen resynthesis, the tissue concn being still lower than the control after 40 min of recovery; (c) a progressive restoration of α-ketoglutarate content with a time-dependent tendency towards elevation of succinate levels at values higher than normal; (d) a normalization of creatine phosphate, ADP and AMP contents, the ATP concns being depressed although there was a tendency of the energy charge toward near-normalization after 40 min of recovery; (e) a decrease in ammonia to normal values, with a time-dependent normalization of glutamate, aspartate and gamma-aminobutyrate contents; (f) a progressive rise in glutamine content, the value being still lower than the control after 40 min; (g) a time-dependent increase in alanine concn with a

Table 6. Motor area of cerebral cortex of beagle dogs: contents of fatty acids, phospholipids and free fatty acids after 10 min of posthypoglycemic recovery (recovery time 10 min) and treatment for 20 plus 10 min with an intracarotid perfusion ($0.1\ ml \cdot kg^{-1} \cdot min^{-1}$) with saline solution or drug solution

	Fatty acids						Phospholipids				
Treatment	Palmitic acid	Stearic acid	Oleic acid	Arachidonic acid	Docosahexenoic acid	Total	Choline phosphoglycerides	Inositol + serine phosphoglycerides	Ethanolamine phosphoglycerides	Total	Free fatty acids
Saline solution	28.1 ± 1.3	24.2 ± 1.9	23.1 ± 1.6	11.9 ± 1.4	21.6 ± 1.6	114.0 ± 2.1	22.8 ± 1.3	11.4 ± 1.6	25.5 ± 1.9	69.5 ± 2.9	1.02 ± 0.05
Papaverine	29.3 ± 2.2	26.0 ± 1.6	24.8 ± 1.8	13.1 ± 2.1	22.4 ± 1.7	118.6 ± 3.4	21.7 ± 2.3	10.6 ± 0.9	23.7 ± 2.1	67.1 ± 1.4	1.00 ± 0.10
Uridine	29.2 ± 1.4	25.6 ± 2.3	24.2 ± 3.9	11.3 ± 1.4	20.7 ± 2.1	112.8 ± 3.0	21.3 ± 2.6	10.6 ± 1.1	27.1 ± 1.9	71.1 ± 5.8	1.07 ± 0.13
Cytidine	25.8 ± 2.3	25.7 ± 1.6	22.0 ± 1.2	12.5 ± 1.1	22.3 ± 1.6	117.1 ± 2.1	25.6 ± 1.6	12.1 ± 0.6	24.3 ± 1.9	67.2 ± 1.7	0.96 ± 0.07
DL-Carnitine	23.9 ± 0.9*	21.2 ± 1.1	19.1 ± 1.1*	10.7 ± 1.3	19.9 ± 3.2	102.3 ± 2.0*	20.5 ± 2.8	9.6 ± 1.7	22.7 ± 2.0	63.2 ± 3.4	0.89 ± 0.10
DL-Acetylcarnitine	23.5 ± 2.2*	20.3 ± 1.3	18.7 ± 2.6*	10.3 ± 1.1	19.7 ± 2.9	99.0 ± 4.6*	20.3 ± 2.2	9.8 ± 1.6	22.4 ± 3.7	62.5 ± 2.6	0.86 ± 0.04

The values are expressed as $\mu moles \cdot (g\ cortex\ wet\ wt)^{-1}$ (means ± S.E.M.).
* = differs from saline solution treated dogs: Anova test ($P < 0.05$): N = 4 (saline solution treated animals) or 3 (drug-treated animals).

Table 7. Motor area of cerebral cortex of beagle dogs: contents of fatty acids, phospholipids and free fatty acids after 20 min of posthypoglycemic recovery (recovery time 20 min) and treatment for 20 plus 20 min with an intracarotid perfusion ($0.1\ ml \cdot kg^{-1} \cdot min^{-1}$) with saline solution or drug solution

	Fatty acids						Phospholipids				
Treatment	Palmitic acid	Stearic acid	Oleic acid	Arachidonic acid	Docosahexenoic acid	Total	Choline phosphoglycerides	Inositol + serine phosphoglycerides	Ethanolamine phosphoglycerides	Total	Free fatty acids
Saline solution	26.1 ± 2.1	21.0 ± 1.0	25.7 ± 2.1	10.5 ± 1.0	22.3 ± 0.9	111.5 ± 4.2	20.9 ± 2.0	10.5 ± 1.2	28.8 ± 2.9	66.3 ± 2.3	0.50 ± 0.08
Papaverine	24.3 ± 2.2	24.0 ± 2.4	23.2 ± 2.7	11.1 ± 0.9	19.8 ± 2.5	110.9 ± 4.2	22.2 ± 2.0	12.1 ± 1.2	26.2 ± 3.0	68.2 ± 3.3	0.62 ± 0.11
Uridine	25.6 ± 2.1	25.6 ± 1.7	26.9 ± 4.6	9.9 ± 1.1	20.0 ± 1.8	112.8 ± 3.7	21.7 ± 2.0	11.2 ± 0.7	29.3 ± 2.2	67.1 ± 2.6	0.46 ± 0.11
Cytidine	27.4 ± 1.8	22.7 ± 1.6	26.2 ± 1.9	12.3 ± 0.8	20.7 ± 1.6	116.7 ± 3.3	19.7 ± 2.8	9.9 ± 0.9	24.7 ± 1.9	64.3 ± 4.3	0.56 ± 0.04
DL-Carnitine	22.5 ± 0.9*	17.4 ± 1.6	21.8 ± 0.9*	8.9 ± 0.4	20.7 ± 1.5	93.5 ± 2.8*	19.4 ± 1.8	9.1 ± 1.0	25.3 ± 1.8	60.5 ± 1.8	0.40 ± 0.04
DL-Acetylcarnitine	22.0 ± 1.1*	17.1 ± 1.4	21.1 ± 1.1*	8.7 ± 0.3	20.9 ± 0.8	91.3 ± 2.9*	19.6 ± 1.8	8.9 ± 0.5	25.9 ± 1.1	61.0 ± 3.3	0.38 ± 0.07

The values are expressed as $\mu moles \cdot (g\ cortex\ wet\ wt)^{-1}$ (means ± S.E.M.).
* = differs from saline solution treated dogs: Anova test ($P < 0.05$): N = 4 (saline solution treated animals) or 3 (drug-treated animals).

Table 8. Motor area of cerebral cortex of beagle dogs: contents of fatty acids, phospholipids and free fatty acids after 40 min of posthypoglycemic recovery (recovery time 40 min) and treatment for 20 plus 40 min with an intracarotid perfusion (0.1 ml·kg^{-1}·min^{-1}) with saline solution or drug solution

Treatment	Fatty acids						Phospholipids				Free fatty acids
	Palmitic acid	Stearic acid	Oleic acid	Arachidonic acid	Docosahexenoic acid	Total	Choline phosphoglycerides	Inositol + serine phosphoglycerides	Ethanolamine phosphoglycerides	Total	
Saline solution	28.3 ± 1.5	22.8 ± 1.3	24.6 ± 1.2	12.3 ± 1.6	22.8 ± 1.5	117.6 ± 1.8	21.7 ± 0.7	11.0 ± 0.8	27.3 ± 1.4	67.9 ± 1.0	0.26 ± 0.02
Papaverine	26.8 ± 2.1	24.7 ± 1.3	23.7 ± 2.1	12.5 ± 1.3	22.5 ± 0.5	119.2 ± 2.7	25.2 ± 1.5	12.2 ± 0.5	30.1 ± 1.6	69.3 ± 1.5	0.31 ± 0.03
Uridine	29.3 ± 2.8	25.0 ± 1.9	26.2 ± 1.9	10.8 ± 0.5	21.4 ± 1.4	115.3 ± 3.1	23.0 ± 1.4	10.3 ± 0.7	29.0 ± 1.6	64.9 ± 3.1	0.30 ± 0.05
Cytidine	30.1 ± 1.6	23.6 ± 1.0	25.2 ± 1.5	11.6 ± 1.0	23.7 ± 1.2	118.9 ± 0.9	22.5 ± 1.2	11.4 ± 0.6	28.9 ± 1.3	65.5 ± 1.7	0.25 ± 0.05
DL-Carnitine	24.9 ± 1.8	19.5 ± 1.3	21.2 ± 1.9*	10.5 ± 1.3	21.1 ± 1.7	99.8 ± 2.2*	20.2 ± 1.6	9.7 ± 1.1	24.2 ± 1.6	63.9 ± 2.1	0.25 ± 0.03
DL-Acetylcarnitine	24.0 ± 1.7	19.1 ± 1.8	20.8 ± 1.1*	10.3 ± 1.4	21.8 ± 1.0	100.3 ± 2.2*	20.6 ± 1.4	9.7 ± 1.1	24.7 ± 1.4	63.1 ± 2.5	0.25 ± 0.04

The values are expressed as μmoles·(g cortex wet wt)$^{-1}$ (means ± S.E.M.).
* = differs from saline solution treated dogs: Anova test ($P < 0.05$): N = 4 (saline solution treated animals) or 3 (drug-treated animals).

tendency towards elevation at values higher than normal after 20 and 40 min of recovery; (h) a progressive and extensive reduction of free fatty acid concns, the values returning to normal 40 min after recovery; and (i) no changes in total or individual fatty acids and phospholipids.

Drug action during hypoglycemia and posthypoglycemic recovery

After 20 min of severe hypoglycemia and intracarotid perfusion with the substances tested (i.e. papaverine, uridine, cytidine, DL-carnitine, DL-acetylcarnitine) the motor area of the cerebral cortex responded (data not shown) with no changes in the contents of the various metabolites evaluated, with the exception of DL-acetylcarnitine which induced a decrease in the concn of total fatty acids [from 113.2 ± 2.1 to 103.8 ± 2.0 μmoles·(g cortex wet wt)$^{-1}$].

During the posthypoglycemic recovery (Tables 3–5), compared to the values from saline solution treated dogs (control animals), the intracarotid perfusion with uridine lowered the cerebral contents of glucose, lactate and pyruvate, and increased the contents of succinate, glutamine and alanine, the ammonia concn being significantly affected only after 20 min of recovery. These effects seem to be unrelated to vasal action because the metabolite values from uridine-treated dogs were always different from the values obtained in papaverine-treated dogs. The only effects of the intracarotid perfusion with cytidine were an increased content of succinate at 10 and 20 min, and an increased concn of alanine after 20 min of posthypoglycemic recovery.

During the posthypoglycemic recovery (Tables 6–8), compared to the values from saline solution treated dogs (control animals), the intracarotid perfusion with both DL-carnitine and DL-acetylcarnitine lowered the cerebral contents of the total fatty acids as well as some individual fatty acids concns (palmitic acid, oleic acid), the free fatty acids being unchanged probably because of their translocation from brain to blood. The effect of DL-carnitine and DL-acetylcarnitine seems to be unrelated to a vasal action because of the fact that the fatty acid values from drug-treated animals were always different from the values obtained from papaverine-treated dogs, papaverine being in any case inactive.

DISCUSSION

In the motor area of the cerebral cortex of beagle dogs, 20 min of severe hypoglycemia (arterial blood glucose level falling below 1 μmole·g^{-1}) induced a depletion of the stores of glucose and glycogen, with a marked reduction in the contents of lactate, pyruvate and α-ketoglutarate. During posthypoglycemic recovery the cortical glucose content returned to normal, the rate of glycogen synthesis being very slow (about 6.25 nmoles·g^{-1}·min^{-1}). Furthermore, there was a tendency towards normalization of pyruvate and α-ketoglutarate contents, although lactate and succinate increased to above normal. Hypoglycemia reduced both ATP and creatine phosphate cortical contents and increased AMP and ADP concns, the energy charge potential being decreased

by about 34%. During posthypoglycemic recovery there was a time-dependent restitution of cortical contents of creatine phosphate, ADP and AMP, while after 40 min of recovery there was a persisting decrease in both ATP content and the adenine nucleotide sum, probably related to deamination and dephosphorylation of AMP to inosine monophosphate and adenosine during hypoglycemia, with translocation of breakdown products from brain to blood [48, 49]. The intracarotid perfusion with the substances tested (uridine, cytidine, carnitine, acetylcarnitine) did not change the trend of the energy mediator modification during the posthypoglycemic recovery.

Severe hypoglycemia induced: (a) an extensive decrease in glutamate, glutamine, alanine and gamma-aminobutyrate; and (b) an increase in aspartate and particularly ammonia. The increase in aspartate may be related to the fall in pyruvate available for acetyl-CoA production and therefore for the synthesis of citrate, with accumulation of oxaloacetate [50, 51]. This event shifts the transamination reaction catalyzed by aspartate aminotransferase to the formation of both aspartate and α-ketoglutarate. Notwithstanding, the content of α-ketoglutarate is reduced probably because of its higher than normal ultilization for both the α-ketoglutarate dehydrogenase reaction (leading to the formation of succinyl-CoA and succinate) and the glutamate dehydrogenase reaction (leading to the formation of glutamate available for the reactions catalyzed by glutamate decarboxylase and alanine aminotransferase). The accumulation of ammonia could be explained by catabolism of adenine nucleotides, oxidation of glutamate, and decreased synthesis of glutamine because of ATP depletion. During the posthypoglycemic recovery there was a tendency toward normalization of the amino acids tested, although the succinate content was higher and the glutamine content was lower than the control after 40 min of restitution.

The intracarotid perfusion with uridine affected some cerebral biochemical parameters during the posthypoglycemic recovery, inducing: (a) an increase in alanine and glutamine cerebral contents; and (b) a decrease in pyruvate concn, suggesting an activation of the transamination and amination reactions. Furthermore, the treatment with uridine caused a decrease in glucose, pyruvate and lactate cerebral contents, and an increase in succinate concn: therefore an activation of the succinate cycle [52, 53] may also be hypothesized. The action induced by the intracarotid perfusion with cytidine was much lower than that induced by uridine, thus confirming previous observations [25–27, 52] that, among pyrimidine nucleosides, uracyl ones are the most active.

Severe hypoglycemia decreased the total phospholipids, and among the individual phospholipids there were decreases in choline phosphoglycerides while the ethanolamine phosphoglycerides and the inositol plus serine phosphoglycerides were not significantly decreased. Among the individual fatty acids, there was a decrease in stearic acid (18:0), oleic acid (18:1) and arachidonic acid (20:4). The free fatty acids concn was increased by about 400% after 20 min of severe hypoglycemia. The level of free fatty acids was normal after 40 min of posthypoglycemic recovery, while the alterations in the individual and total phospholipids and fatty acids persisted. During posthypoglycemic recovery, the intracarotid perfusion with DL-carnitine and DL-acetylcarnitine induced a magnification of the decrease in the individual and total fatty acids.

This action may be related to the fact that carnitine acts as a cofactor for the oxidation of fatty acids, the acyl portions of acylcarnitines (unlike those of acyl-CoA esters) being readily oxidized by mitochondria [28, 29]. The stimulation of mitochondrial oxidation of fatty acids by carnitine requires the activity of mitochondrial carnitine acetyltransferase, but the carnitine-dependent transport of acyl groups requires the operation of a translocase system catalyzing a mole-to-mole exchange diffusion of carnitine and acylcarnitines [54]. The rate of this translocase may control the rate of the carnitine-dependent transport of fatty acids into mitochondria and, therefore, may control the final fatty acid oxidation. The observation that an increase in tissue and intramitochondrial carnitine enhances the rate of carnitine–acylcarnitine translocase catalyzed transport [54–57] suggests that the rate of translocase reaction may be limited by the actual concn of carnitine in the matrix during the hypoglycemic injury and the posthypoglycemic recovery. In this instance, the carnitine or acetylcarnitine intracarotid perfusion may enhance the pool size of intramitochondrial carnitine and/or acylcarnitines contributing to the enhanced ability to oxidize fatty acids, as indicated by the larger reduction in the fatty acid content of the cerebral cortex during the posthypoglycemic reovery.

The complete oxidation of fatty acids of the chain length observed in the present research plus the oxidation of the glycerol moiety from phospholipids would obviously cover more than all the non-glucose consumption. On the other hand, no preferential loss of polyenoic fatty acids (20:4 and 22:6) and ethanolamine phosphoglycerides [58] was found in the present research and, therefore, an important peroxidative degradation of tissue lipid may be ruled-out, corroborating those previously reported by others [5]. Although during posthypoglycemic recovery the magnification of the fatty acid reduction by carnitine and acetylcarnitine may provide other substances for oxidation, this hypothesis is hampered by the fact that this event is still present also during the extensive restoration of cerebral glycolytic metabolites. In this instance a question can be raised concerning how fatty acids are utilized by the carnitine-treated brain during posthypoglycemic recovery, although it cannot be excluded that lipid oxidation may represent a cerebral mechanism of sparing action for other oxidative substrates. Our continued work is directed towards this problem by evaluating the interference of some active forms of carnitine (e.g., L-acetylcarnitine) with the enzymatic activities related to cerebral energy transduction.

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