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INFLUENCE OF AGING AND EXOGENOUS SUBSTANCES ON CEREBRAL ENERGY METABOLISM IN POSTHYPOGLYCEMIC RECOVERY

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Abstract—In rats of different ages, acute severe hypoglycemia with isoelectric EEG induced extensive deterioration of the energy state and gross alteration of amino acid contents. During recovery of adult animals, tissue glucose concentration returned to normal, while the rate of glycogen synthesis was slow, both lactate and pyruvate concentrations increasing above normal. In the recovery period of "adult" rats, the ATP concentration increased but the adenine nucleotide pool remained reduced, even if the ADP and AMP concentrations were close to normal. Phosphocreatine was restored to normal concentrations with reciprocal changes in creatine content. In adult rats, during the recovery there was a rise in glutamate and glutamine concentrations, gamma-aminobutyrate concentration returning to normal value. Ammonia and aspartate decreased below normal, while alanine increased above normal. Aging does not affect the cerebral metabolic derangement occurring in severe hypoglycemia, but rather the metabolic changes that the brain tends to reverse during the posthypoglycemic restitution. In fact, there was lower restitution of the concentrations of cerebral cortical metabolites of "mature" and "senescent" rats in comparison with "adult" ones. Particularly, in older brains the concentrations of many amino acids and adenylate nucleotides remained largely abnormal.

The effect of some agents on the posthypoglycemic recovery was tested: (a) dihydroergocristine; (b) eburnamonine; (c) raubasine; (d) almitrine; (e) piracetam. During the posthypoglycemic recovery, these different agents exhibited different interferences on glycolytic metabolites, amino acids and energy-rich phosphates. However, a more limited effect of the tested agents, which decrease with aging, was observed.

Severe hypoglycemia induces gross derangement of cerebral energy state, with reduced concentrations of ATP and phosphocreatine and with increased contents of ADP, AMP and creatine. With the degree of hypoglycemia achieved in the present study (<1.0 μmole/g), glucose transport into the brain is rate-limiting [1, 2] and insufficient to support brain energy metabolism. The observation that the CMR glucose declines at a faster rate with decreasing plasma glucose than does the CMR oxygen, suggests oxidation of other endogenous substrates including glycolytic intermediates as well as Krebs cycle intermediates and related amino acids [3–13]. During posthypoglycemic restitution by glucose administration there is a moderate recovery of cerebral energy state. However, a persisting reduction in the pool size of both adenine nucleotides and amino acids occurs [4, 6–8].

The above-quoted data were obtained from "adult" animals, without paying any attention to eventual changes related to aging. It is therefore important to investigate at cerebral level the effect of aging on the biochemical modifications induced by posthypoglycemic recovery following acute severe hypoglycemia. On the other hand, the posthypoglycemic recovery could be a suitable model for the evaluation of the influence of exogenous agents on brain substrate utilization in an experimental condition characterized by a derangement in carbohydrate and amino acid metabolism without disruption of the oxygen supply. In this way, there exist agents widely used clinically in humans, which are classified as cerebral "vasoactive", "metabolic" or "vasculometabolic" substances. Clearly this classification is a vague and superficial one. On the other hand, this work is intended also to overcome it, besides being aimed at detecting the action of these substances on several metabolites related to cerebral energy metabolism during the posthypoglycemic recovery. The following agents were chosen: dihydroergocristine; eburnamonine; raubasine; almitrine and piracetam.

Therefore, the aim of the present study was to evaluate the changes induced by aging on cerebral energy metabolism in severe hypoglycemia and during the posthypoglycemic recovery with or without treatment with the above-quoted substances. Rats of different ages (20, 60 and 100 weeks of age) were made hypoglycemic by insulin injection and maintained with an isoelectric EEG for 20 min. Recovery was induced for 20 min by glucose injection. The brain cortical concentrations of carbohydrates, amino acids and energy mediators were evaluated.

MATERIALS AND METHODS

Animals and blood sampling technique. The experiments were performed on male Sprague–Dawley rats 20, 60 or 100 weeks old, roughly cor-

responding to "adult", "mature" and "senescent" animals. Obviously, these attributions are totally arbitrary and imprecise. However, they are used to facilitate the singling out of certain ages during rats' life. The rats were fasted 24 hr before the experiments but had free access to water. Animals were curarized with tubocurarine chloride (0.4–0.6 mg/kg, i.v.) and artificially ventilated with 70% N_2O and 30% O_2. Body temperature was maintained close to 37.2°. Blood samples from one femoral artery were anaerobically taken for the determination of pH and of CO_2 and O_2 partial pressure, or collected directly in liquid nitrogen for the measurement of glucose level. A plastic funnel was accommodated over the skull bone for later freezing of the brain *in situ* with liquid nitrogen. One hour before the anesthesia and preparative procedure, insulin (40 I.U./kg in 2 ml/kg of Krebs–Henseleit solution) was injected i.p. The beginning of the period of severe hypoglycemia was defined as the time when spontaneous EEG activity disappeared.

Experimental plan and withdrawal of cerebral samples. The following groups of rats were used. Three control groups of 10 "adult", 6 "mature" and 6 "senescent" sham-operated rats were maintained for about 3 hr before brain tissue was frozen *in situ* by liquid nitrogen. In the other groups, animals were kept at steady state until the EEG became isoelectric after a period of about 3 hr from insulin injection. Three groups of 6 "adult", 6 "mature", and 6 "senescent" rats were kept hypoglycemic and maintained for 20 min with the isoelectric EEG before tissue was sampled. Other groups of "adult", "mature", and "senescent" rats (N = 6 in each) were allowed to recover for 20 min following the isoelectric period of 20 min before brain tissue was frozen *in situ* by liquid nitrogen (posthypoglycemic recovery). Recovery was induced by glucose i.v. injection (1 ml/kg of a 50% glucose solution) and maintained by glucose i.v. perfusion.

Therefore, in the cerebral cortex of "adult" or "mature" or "senescent" rats, metabolites were measured: (a) in control condition; (b) after 20 min of severe hypoglycemia; (c) after 20 min of posthypoglycemic recovery following an isoelectric EEG period of 20 min. At the end of a 20-min period of posthypoglycemic recovery the effect of an i.v. perfusion with some agents was tested. The zero time for the beginning of the treatment was defined as the time of onset of isoelectric EEG, the perfusion being therefore of 40 min duration, at the rate of 0.02 ml/min per kg with (a) saline solution, (b) dihydroergocristine (1×10^{-3} M) (c) eburnamonine (4×10^{-2} M), (d) raubasine (3×10^{-3} M) (e) almitrine (5×10^{-3} M), (f) piracetam (8×10^{-1} M). Before the experiment, the animals were pretreated for two weeks (6 days a week) with i.p. injections (0.8 ml/kg) of the same agents at the above-quoted molar concentrations.

Analytical technique. For the analysis of carbohydrates, amino acids and energy mediators, the brains were frozen *in situ* by liquid nitrogen. Brain cortical tissue from fronto-parietal region was extracted at −20° with HCl-methanol and subsequently at 0° with perchloric acid. The neutralized perchloric acid free extracts were analysed [14, 15] for: glycogen, glucose, lactate, pyruvate; glutamate, glutamine, aspartate, alanine, GABA, ammonia; ATP, ADP, AMP, phosphocreatine, creatine. The energy state of the cortical cerebral tissue was evaluated in terms of the adenylate energy charge, calculated as: $([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP])$.

Statistical analysis. The analysis of variance (ANOVA) for factorial experiment was applied ($P < 0.01$).

RESULTS

Cerebral changes by severe hypoglycemia in animals of different ages

In the cerebral cortex of rats of the different ages tested (Tables 1–3), a 20-min period of severe hypoglycemia with isoelectric EEG induced an extensive breakdown of creatine phosphate and ATP, while ADP, AMP and creatine increased, the behaviour being unaffected by aging. Furthermore, severe hypoglycemia decreased the cortical concentrations of glycolytic metabolites: glycogen, glucose, pyruvate, lactate. A massive accumulation of cerebral ammonia was associated to a reduction of glutamate, glutamine, alanine and GABA, and to an increase in aspartate cortical concentrations, the behaviour being age-independent from a biological point of view.

Cerebral age-related changes during posthypoglycemic recovery

In "adult" animals (Table 1), after 20 min of posthypoglycemic recovery, cerebral phosphocreatine was restored to normal, while in both "mature" and "senescent" rats (Tables 2 and 3) the values were lower than normal. The cortical ATP concentration, the energy charge potential and the adenine nucleotide pool size were still lower than normal after 20 min of posthypoglycemic recovery, the metabolic state being more largely abnormal in "senescent" animals. There was restoration of brain glucose concentration, the little glycogen resynthesis being slower in older brains. Furthermore, pyruvate cortical concentrations rose above control in both "adult" and "mature" rats (Tables 1 and 2), while in "senescent" animals (Table 3) its concentration after 20 min of recovery was lower than normal. Lactate cerebral concentration increased above normal in rats of different ages, with greater values in older brains.

After 20 min of recovery there was a tendency towards elevation of glutamate and glutamine concentrations, but the values were still lower than normal particularly in "mature" and "senescent" rat brains. Alanine increased above normal during the posthypoglycemic period in the cortical tissues of rats of different ages. Aspartate, gamma-aminobutyrate and ammonia returned to normal or near-normal values in the cerebral cortex of "adult" and "mature" rats (Tables 1 and 2), while in "senescent" animals (Table 3) their values were greater (ammonia) or lower (aspartate, gamma-aminobutyrate) than normal after 20 min of recovery. As a result, in comparison with the control values, the cerebral free amino acid pool remained reduced after 20 min of recovery, the reduction being by about 4.75 in

Table 1. Adult rat: cerebral cortical contents of carbohydrate substrates, labile phosphates, amino acids and ammonia in the recovery period (20 min) following severe hypoglycemia with isoelectric EEG (for 20 min) and i.v. infusion (0.02 ml/min per kg) with some drugs (for 20 plus 20 min)

Metabolite or ratio	Controls (a)	Hypoglycemia with an isoelectric EEG for 20 min (b)	Isoelectric EEG for 20 min followed by recovery (20 min). Treatment with:					
			Saline solution (c)	Dihydroergocristine 1×10^{-3} M (d)	Eburnamonine 4×10^{-2} M (e)	Raubasine 3×10^{-3} M (f)	Almitrine 5×10^{-3} M (g)	Piracetam 8×10^{-1} M (h)
Glycogen	2.98 ± 0.07	0.09 ± 0.01*	0.28 ± 0.02*	0.29 ± 0.02	0.31 ± 0.02	0.32 ± 0.02	0.32 ± 0.03	0.36 ± 0.06
Glucose	2.86 ± 0.06	0.13 ± 0.02*	2.70 ± 0.06	2.23 ± 0.09†	2.22 ± 0.08†	2.26 ± 0.06†	2.85 ± 0.05	2.86 ± 0.12
Pyruvate	0.112 ± 0.004	0.023 ± 0.002*	0.181 ± 0.004*	0.138 ± 0.006†	0.158 ± 0.006	0.147 ± 0.008†	0.172 ± 0.007	0.191 ± 0.010
Lactate	1.56 ± 0.05	0.46 ± 0.02*	2.55 ± 0.07*	3.51 ± 0.09†	3.16 ± 0.08†	2.45 ± 0.10	2.50 ± 0.07	2.53 ± 0.10
Lactate/pyruvate	14.17 ± 0.79	20.54 ± 1.69*	14.09 ± 0.61	25.66 ± 1.43†	20.11 ± 1.09†	16.71 ± 0.63	14.64 ± 0.64	13.40 ± 0.99
Glutamate	11.82 ± 0.40	2.82 ± 0.06*	9.71 ± 0.25	10.35 ± 0.29	9.74 ± 0.12	8.23 ± 0.11†	9.43 ± 0.25	7.68 ± 0.11†
Glutamine	4.85 ± 0.05	0.46 ± 0.03*	2.58 ± 0.11*	3.07 ± 0.05	3.95 ± 0.05†	2.38 ± 0.10	2.89 ± 0.10	2.83 ± 0.09
Aspartate	3.91 ± 0.06	16.25 ± 0.84*	3.50 ± 0.14	3.03 ± 0.07	3.55 ± 0.11	3.06 ± 0.06	3.75 ± 0.12	4.72 ± 0.11†
Alanine	0.502 ± 0.010	0.215 ± 0.006*	0.830 ± 0.025*	0.705 ± 0.022†	0.722 ± 0.007†	0.850 ± 0.012	0.864 ± 0.013	0.979 ± 0.013†
γ-Aminobutyrate	2.36 ± 0.08	0.86 ± 0.03*	2.07 ± 0.07	1.97 ± 0.06	2.01 ± 0.07	2.37 ± 0.04	1.93 ± 0.10	1.90 ± 0.08
Ammonia	0.35 ± 0.02	2.95 ± 0.07*	0.35 ± 0.02	0.30 ± 0.03	0.28 ± 0.02	0.34 ± 0.03	0.30 ± 0.03	0.32 ± 0.03
Amino acid pool size	23.44 ± 0.72	20.60 ± 0.48*	18.69 ± 0.43*	19.12 ± 0.51	19.97 ± 0.39	16.89 ± 0.57	18.86 ± 0.75	18.11 ± 0.68
ATP	2.81 ± 0.03	0.71 ± 0.03*	2.01 ± 0.03*	2.36 ± 0.03†	2.35 ± 0.05†	2.07 ± 0.04	2.10 ± 0.06	2.28 ± 0.02†
ADP	0.32 ± 0.02	1.26 ± 0.04*	0.40 ± 0.02	0.30 ± 0.02	0.29 ± 0.01†	0.34 ± 0.01	0.35 ± 0.03	0.26 ± 0.01†
AMP	0.06 ± 0.01	0.48 ± 0.02*	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01
Adenine nucleotide pool size	3.18 ± 0.03	2.45 ± 0.04*	2.50 ± 0.05*	2.75 ± 0.04	2.71 ± 0.05	2.49 ± 0.05	2.54 ± 0.06	2.64 ± 0.03
PCr	4.62 ± 0.08	0.90 ± 0.04*	4.31 ± 0.12	3.68 ± 0.07†	4.12 ± 0.12	4.54 ± 0.11	4.72 ± 0.08	4.47 ± 0.13
Creatine	6.02 ± 0.09	8.98 ± 0.48*	6.56 ± 0.20	7.39 ± 0.15	6.57 ± 0.21	6.56 ± 0.12	6.10 ± 0.09	6.30 ± 0.08
E.C.P.	0.931 ± 0.003	0.548 ± 0.005*	0.883 ± 0.004*	0.913 ± 0.009	0.923 ± 0.004†	0.900 ± 0.006	0.895 ± 0.009	0.914 ± 0.004†
No. of animals	10	6	6	6	6	6	6	6

The values are given in μmoles/g wet weight and are expressed as means ± S.E.M.

ANOVA test ($P < 0.01$): * controls (a) vs hypoglycemic rats (b) or saline-treated posthypoglycemic rats (c); † saline-treated posthypoglycemic rats (c) vs drug-treated rats (d to h).

PCr, phosphocreatine; E.C.P., energy charge potential.

Table 2. Mature rat: cerebral cortical contents of carbohydrate substrates, labile phosphates, amino acids and ammonia in the recovery period (20 min) following severe hypoglycemia with isoelectric EEG (for 20 min) and i.v. infusion (0.02 ml/min per kg) with some drugs (for 20 plus 20 min)

Metabolite or ratio	Controls (a)	Hypoglycemia with an isoelectric EEG for 20 min (b)	Isoelectric EEG for 20 min followed by recovery (20 min). Treatment with: Saline solution (c)	Dihydroergocristine 1×10^{-3} M (d)	Eburnamonine 4×10^{-2} M (e)	Raubasine 3×10^{-3} M (f)	Almitrine 5×10^{-3} M (g)	Piracetam 8×10^{-1} M (h)
Glycogen	3.04 ± 0.10	0.07 ± 0.01*	0.20 ± 0.02*	0.16 ± 0.03	0.22 ± 0.02	0.23 ± 0.03	0.28 ± 0.03	0.25 ± 0.04
Glucose	2.63 ± 0.08	0.09 ± 0.01*	2.58 ± 0.12	2.19 ± 0.06†	2.28 ± 0.05†	2.12 ± 0.07†	1.96 ± 0.06†	2.77 ± 0.13
Pyruvate	0.121 ± 0.007	0.017 ± 0.002*	0.153 ± 0.008*	0.138 ± 0.012	0.146 ± 0.011	0.122 ± 0.002†	0.141 ± 0.008	0.168 ± 0.013
Lactate	1.81 ± 0.09	0.39 ± 0.03*	3.02 ± 0.13*	3.75 ± 0.09†	3.57 ± 0.14†	3.26 ± 0.25	2.81 ± 0.15	2.88 ± 0.15
Lactate/pyruvate	14.96 ± 0.83	20.53 ± 1.80*	20.14 ± 1.98*	28.12 ± 2.27†	25.18 ± 2.17	25.57 ± 2.52	20.20 ± 1.51	17.59 ± 1.44
Glutamate	11.23 ± 0.37	2.57 ± 0.09*	8.58 ± 0.22*	8.87 ± 0.28	8.32 ± 0.17	7.59 ± 0.08†	8.16 ± 0.26	8.09 ± 0.43
Glutamine	4.70 ± 0.07	0.42 ± 0.01*	2.06 ± 0.10*	2.39 ± 0.14	2.74 ± 0.06†	2.18 ± 0.16	2.28 ± 0.09	1.95 ± 0.18
Aspartate	3.72 ± 0.10	15.78 ± 0.72*	3.42 ± 0.20	3.12 ± 0.19	3.31 ± 0.16	3.21 ± 0.27	3.65 ± 0.20	4.18 ± 0.17†
Alanine	0.525 ± 0.017	0.201 ± 0.012*	0.725 ± 0.023*	0.672 ± 0.023	0.677 ± 0.018	0.772 ± 0.025	0.762 ± 0.018	0.833 ± 0.019†
γ-Aminobutyrate	2.23 ± 0.14	0.80 ± 0.02*	1.79 ± 0.07	1.61 ± 0.15	1.86 ± 0.12	1.68 ± 0.14	1.59 ± 0.14	1.67 ± 0.10
Ammonia	0.38 ± 0.03	3.12 ± 0.10*	0.39 ± 0.02	0.35 ± 0.04	0.35 ± 0.03	0.37 ± 0.05	0.32 ± 0.02	0.36 ± 0.04
Amino acid pool size	22.40 ± 0.68	19.77 ± 0.75*	16.57 ± 0.74*	16.66 ± 0.68	16.91 ± 0.54	15.43 ± 0.56	16.44 ± 0.73	16.72 ± 0.81
ATP	2.73 ± 0.04	0.67 ± 0.03*	1.77 ± 0.05*	2.02 ± 0.04†	1.99 ± 0.03†	1.81 ± 0.08	1.82 ± 0.06	2.00 ± 0.04†
ADP	0.36 ± 0.02	1.28 ± 0.05*	0.46 ± 0.03	0.39 ± 0.02	0.38 ± 0.03	0.42 ± 0.05	0.42 ± 0.03	0.33 ± 0.02†
AMP	0.05 ± 0.01	0.51 ± 0.02*	0.15 ± 0.02*	0.18 ± 0.02	0.12 ± 0.02	0.16 ± 0.02	0.13 ± 0.02	0.16 ± 0.01
Adenine nucleotide pool size	3.14 ± 0.04	2.46 ± 0.05*	2.38 ± 0.05*	2.59 ± 0.05	2.49 ± 0.04	2.36 ± 0.07	2.37 ± 0.05	2.49 ± 0.04
PCr	4.49 ± 0.10	0.87 ± 0.05*	3.70 ± 0.13*	3.21 ± 0.16†	3.52 ± 0.11	3.85 ± 0.28	3.98 ± 0.34	3.98 ± 0.15
Creatine	6.51 ± 0.12	9.65 ± 0.36*	7.02 ± 0.45	7.24 ± 0.37	7.13 ± 0.32	6.71 ± 0.17	6.00 ± 0.26	6.17 ± 0.17
E.C.P.	0.926 ± 0.004	0.532 ± 0.004*	0.840 ± 0.004*	0.855 ± 0.005	0.875 ± 0.006†	0.846 ± 0.008	0.858 ± 0.010	0.868 ± 0.005
No. of animals	10	6	6	6	6	6	6	6

The values are given in μmoles/g wet weight and are expressed as means ± S.E.M.

ANOVA test ($P < 0.01$): * controls (a) vs hypoglycemic rats (b) or saline-treated posthypoglycemic rats (c); † saline-treated posthyglycemic rats (c) vs drug-treated rats (d to h).

PCr = phosphocreatine; E.C.P. = energy charge potential.

Table 3. Senescent rat: cerebral cortical contents of carbohydrate substrates, labile phosphates, amino acids and ammonia in the recovery period (20 min) following severe hypoglycemia with isoelectric EEG (for 20 min) and i.v. infusion (0.02 ml/min per kg) with some drugs (for 20 plus 20 min)

Metabolite or ratio	Controls (a)	Hypoglycemia with an isoelectric EEG for 20 min (b)	Isoelectric EEG for 20 min followed by recovery (20 min). Treatment with: Saline solution (c)	Dihydroergocristine 1×10^{-3} M (d)	Eburnamonine 4×10^{-2} M (e)	Raubasine 3×10^{-3} M (f)	Almitrine 5×10^{-3} M (g)	Piracetam 8×10^{-1} M (h)
Glycogen	2.73 ± 0.11	0.07 ± 0.02*	0.13 ± 0.03*	0.10 ± 0.02	0.14 ± 0.02	0.15 ± 0.03	0.18 ± 0.02	0.10 ± 0.01
Glucose	2.59 ± 0.10	0.08 ± 0.02*	3.05 ± 0.15	2.61 ± 0.09†	2.70 ± 0.05†	2.87 ± 0.17	2.50 ± 0.09†	3.19 ± 0.25
Pyruvate	0.124 ± 0.005	0.012 ± 0.001*	0.100 ± 0.007*	0.097 ± 0.010	0.108 ± 0.005	0.104 ± 0.005	0.102 ± 0.005	0.117 ± 0.004
Lactate	1.88 ± 0.06	0.36 ± 0.02*	3.52 ± 0.13*	4.38 ± 0.13†	4.03 ± 0.08†	3.36 ± 0.17	2.91 ± 0.09†	3.41 ± 0.19
Lactate/pyruvate	15.16 ± 0.90	21.18 ± 1.82*	32.65 ± 2.53*	47.35 ± 5.19†	37.64 ± 2.03	32.75 ± 2.64	28.87 ± 1.52	29.54 ± 2.48
Glutamate	11.10 ± 0.52	2.28 ± 0.10*	7.80 ± 0.22*	8.19 ± 0.28	7.66 ± 0.14	7.02 ± 0.09†	7.26 ± 0.27	7.41 ± 0.33
Glutamine	4.53 ± 0.08	0.39 ± 0.01*	1.63 ± 0.18*	1.83 ± 0.21	1.89 ± 0.12	1.53 ± 0.13	2.00 ± 0.09†	1.80 ± 0.12
Aspartate	3.52 ± 0.09	15.62 ± 0.91*	2.95 ± 0.13*	2.68 ± 0.19	3.05 ± 0.22	2.70 ± 0.11	3.06 ± 0.11	3.18 ± 0.25
Alanine	0.542 ± 0.020	0.192 ± 0.014*	0.652 ± 0.016*	0.617 ± 0.031	0.603 ± 0.023	0.676 ± 0.025	0.684 ± 0.027	0.760 ± 0.011†
γ-Aminobutyrate	2.17 ± 0.09	0.73 ± 0.03*	1.47 ± 0.12*	1.38 ± 0.15	1.56 ± 0.10	1.56 ± 0.10	1.39 ± 0.08	1.39 ± 0.07
Ammonia	0.37 ± 0.03	3.20 ± 0.12*	0.52 ± 0.07*	0.47 ± 0.05	0.45 ± 0.04	0.54 ± 0.05	0.32 ± 0.02†	0.47 ± 0.04
Amino acid pool size	21.86 ± 0.65	19.21 ± 0.59	14.50 ± 0.38*	14.70 ± 0.53	14.76 ± 0.47	13.49 ± 0.35	14.39 ± 0.61	14.54 ± 0.43
ATP	2.65 ± 0.02	0.63 ± 0.02*	1.34 ± 0.08*	1.56 ± 0.05†	1.52 ± 0.04†	1.37 ± 0.02	1.41 ± 0.03	1.53 ± 0.03†
ADP	0.38 ± 0.04	1.32 ± 0.03*	0.46 ± 0.03*	0.40 ± 0.01	0.39 ± 0.02	0.50 ± 0.04	0.41 ± 0.03	0.38 ± 0.02†
AMP	0.05 ± 0.00	0.55 ± 0.02*	0.40 ± 0.04*	0.43 ± 0.03	0.38 ± 0.03	0.38 ± 0.01	0.37 ± 0.04	0.40 ± 0.02
Adenine nucleotide pool size	3.08 ± 0.03	2.50 ± 0.04*	2.20 ± 0.04*	2.39 ± 0.05	2.29 ± 0.04	2.25 ± 0.03	2.19 ± 0.03	2.31 ± 0.03
PCr	4.32 ± 0.12	0.78 ± 0.03*	3.29 ± 0.11*	2.79 ± 0.06†	3.07 ± 0.13	3.39 ± 0.17	3.42 ± 0.25	3.12 ± 0.15
Creatine	6.62 ± 0.08	9.90 ± 0.51*	7.09 ± 0.31	6.85 ± 0.31	6.95 ± 0.40	6.06 ± 0.23	6.15 ± 0.39	6.85 ± 0.31
E.C.P.	0.922 ± 0.003	0.515 ± 0.004*	0.709 ± 0.021*	0.727 ± 0.020	0.749 ± 0.006	0.719 ± 0.006	0.737 ± 0.015	0.743 ± 0.007
No. of animals	10	6	6	6	6	6	6	6

The values are given in μmoles/g wet weight and are expressed as means ± S.E.M.

ANOVA test ($P < 0.01$): * controls (a) vs hypoglycemic rats (b) or saline-treated posthypoglycemic rats (c); † saline-treated posthypoglycemic rats (c) vs drug-treated rats (d to h).

CPr = phosphocreatine; E.C.P. = energy charge potential.

"adult", 5.83 in "mature" and 7.36 μmoles/g in "senescent" rats, respectively.

Exogenous agent effects during posthypoglycemic recovery

The influence of the i.p. pretreatment and the subsequent i.v. infusion with some agents was tested after a 20-min period of recovery. Only those data are given which not only are statistically significant (Tables 1–3) but also of sufficient magnitude to have any biological meaning.

In comparison with saline-treated rats, after the recovery following hypoglycemia and treatment with dihydroergocristine the glucose concentrations decreased in the cerebral cortex of rats of the different ages tested, while pyruvate decreased only in "adult" animals. Since there was always an increase in cortical lactate concentrations, the lactate: pyruvate ratios increased, the increase being greater in older brains. Furthermore, dihydroergocristine induced an increase of ATP concomitant with a decrease in creatine phosphate, the effects being lower in older brains. In the cerebral cortex of rats of the different ages tested, the treatment with eburnamonine induced a decrease in glucose concomitant with a moderate increase in lactate. The glutamine cortical concentrations were increased by eburnamonine in both "adult" and "mature" animals, the amino acid concentration being unaffected in older brains. The agent induced an increase in ATP cerebral concentrations in the rats of the different ages tested, while the increase in the energy charge potential was induced in "adult" and "mature" but not in "senescent" animals.

In comparison with saline-treated rats of the different ages tested, during the posthypoglycemic recovery there was a decrease of cortical glucose and pyruvate concentrations after the treatment with raubasine in "adult" and "mature" but not in "senescent" animals. Furthermore, in raubasine-treated rats of the ages tested there was a tendency towards the decrease of the cortical concentrations of glutamate. In comparison with saline-treated posthypoglycemic animals, almitrine was ineffective on cortical metabolites in "adult" rats, while in "senescent" rats the substance decreased both the glucose and lactate concentrations, and induced an increase in glutamine concomitant with a decrease of ammonia.

In the posthypoglycemic rats piracetam induced: (a) a further increase of alanine above values of saline-treated posthypoglycemic animals of all the ages tested; (b) an increase of aspartate in "adult" and "mature" but not in "senescent" rats; (c) a decrease of glutamate in "adult" animals only. After piracetam treatment, ATP increased above while ADP cortical concentrations decreased below the values of saline-treated rats of the different ages tested.

DISCUSSION

In the cerebral cortex of rats of the different ages tested, severe hypoglycemia induces a similar marked reduction in ATP and creatine phosphate associated with increase in both ADP and AMP. Furthermore, the cerebral cortical stores of glycogen and glucose are almost depleted with an extensive decrease in cerebral pyruvate and lactate. Severe hypoglycemia induces a marked derangement on the cortical free amino acids: decrease of glutamate, glutamine, alanine and gamma-aminobutyrate concomitant with increase of aspartate and ammonia. The decrease of alanine may be related to the fall in pyruvate available for the reaction catalysed by alanine aminotransferase. 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 [9, 10]. This event shifts the transamination reaction catalysed by aspartate aminotransferase to the formation of aspartate. The accumulation of ammonia could be explained by catabolism of adenine nucleotides, oxidation of glutamate, and decreased synthesis of glutamine because of ATP depletion. In any case, the alterations on the cortical metabolites tested are similar in the brains from animals of different ages. Therefore, aging does not seem to affect the type and extent of the cerebral biochemical derangement caused by severe hypoglycemia.

Really, the present results indicate lower restitution of cortical metabolite concentrations in the recovery period after extensive glucose deprivation in older rats. In these animals the incomplete restitution of cerebral energy state is evident from the labile phosphates evaluation and from the fact that lower activity occurred of ATP-requiring reactions (e.g. glycogen and glutamine resynthesis). In fact, after 20 min of recovery in "adult" rats the creatine phosphate cortical concentration is close to normal, while the ATP concentration and the adenine nucleotide pool size remain still reduced, aging increasing this lingering alteration. The event could be also related to the resynthesis of nucleotides occurring by *de novo* synthesis or by salvage pathway. However, this interpretation could be hampered by the fact that (a) the *de novo* synthesis is a very slow process [16] and (b) there is no evidence that the adenine nucleotide pool size increased above hypoglycemia during the recovery period in "adult" or "mature" rats, even if this behaviour is evident for ATP. Therefore, a more likely explanation may be related to a faster purine catabolism or to the fact that the breakdown products (i.e. IMP, inosine and adenosine) accumulated during hypoglycemia are converted to AMP and GMP in the posthypoglycemic period by an age-dependent mechanism.

The quoted reduction in both ATP and adenine nucleotide pool size does not reflect an age-dependent irreversible bioenergetic state. In fact, dihydroergocristine (alpha-blocking ergot alkaloid increasing the CMR glucose and oxygen), eburnamonine (Vinca minor alkaloid increasing the oxygen availability and the CMR glucose and oxygen) and piracetam (nootropic drug enhancing both cerebral molecular cell function and microcirculation) induce a greater increase of ATP cortical concentrations. By some agents (e.g. dihydroergocristine), this event is consistent with an activation of the creatine kinase reaction induced by the higher concentration of cerebral lactate, and resulting in a decrease of the creatine phosphate. The interference

with the labile phosphates can be concomitant with an interference on amino acid (e.g. by piracetam) or on carbohydrate metabolism (e.g by dihydroergocristine and eburnamonine). On the other hand, the interference on carbohydrate and amino acid metabolism may be independent of interference on the energy state (e.g. by raubasine, a tranquilizer and antihypertensive alkaloid from Rauwolfia serpentina).

During the posthypoglycemic recovery, in the cortical cortex of saline-treated "adult" animals glutamate, aspartate and gamma-aminobutyrate tend to normal values, while in older rats there is a markedly lower normalization of the above-quoted amino acids and of glutamine. As a result, the free amino acid pool remains largely more reduced in older brains. Some agents (e.g. eburnamonine and almitrine) induce an increase of glutamine cortical concentrations, sometimes associated with a decrease of ammonia content (e.g. by almitrine). The event may be present only in "adult" and "mature" treated rats (e.g. by eburnamonine) or only in older treated rats (e.g. by almitrine). Probably, this behaviour is related to the power of the agent to enhance the oxygen availability for tissues [17–19], resulting in an interference on the oxidative metabolism in the older brains.

An influence on the transamination processes may be supported by some agents (e.g. by piracetam) resulting in an increase of alanine and aspartate cortical concentrations. The higher values of alanine may be related to an interference on the transamination reaction catalysed by alanine aminotransferase, occurred in the presence of a large pyruvate availability. The higher values of aspartate may be related to the lingering activation of transamination reaction catalysed by aspartate aminotransferase, occurred independently of the glutamate availability. In fact, the changes on transamination reactions may be unrelated to the changes in glutamate concentrations because: (a) in piracetam-treated rats the decrease in the quoted amino acid was present in "adult" rats, while the increase in both alanine and aspartate was also present in "senescent" and/or "mature" rats; (b) in raubasine-treated rats of the different ages tested, the decrease in glutamate unaffected aspartate and alanine cortical concentrations.

In conclusion, the present results show also in older animals an influence of the exogenous treatments on cerebral carbohydrates, amino acids and energy mediators. However, the more limited effects of the tested agents, which decrease with aging, suggests a narrower interference with the mechanisms which underlie the aging. Furthermore, the metabolic intervention only in older brains of an agent increasing the oxygen partial pressure suggests that the cerebral oxygen availability could be decreased as function of aging.

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